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THE FUNCTION OF THE BODY AND FACTORS OF SPACE FLIGHT

Edited by

N. N. Gurovskiy

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ANNOTATION

This monograph is based on data from five years of joint work by leading specialists from socialist countries in space biology and medicine.

The book consists of three sections. The section "Space Physiology" illustrates various aspects of the creation of an artificial cabin atmosphere, the maintenance of heat balance of persons in individual protective equipment, hypodynamia to simulate weightlessness, as well as finding means to increase vestibular stability and preserve spatial orientation during space flights.

The "Space Radiobiology" section discusses radiation safety during space flights. The biological effect of heavy ions was studied on various biological objects, results of biochemical and immunological tests on animals during chronic radiation for three years are given, and experimental data characterizing the temporal organization of cell reproduction in the body are presented; the creation of experimental models of tissue-equivalent dummies with movable joints corresponding to parameters of a "standard man" are discussed.

The third section, "Pharmaco-chemical Protection against Ionizing Radiation," contains data of experimental studies justifying the use in space flight of biological protective means against ionizing radiation which can increase radioresistance and general resistance of the body. For the first time proofs are presented of the possibility of drug prevention of radiation injury caused by prolonged radiation during solar flares.

The book is written for a broad range of specialists working in the area of space biology and medicine and related fields.

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FOREWORD

Because of the continuous development of cosmonautics, space medicine is /4* faced with ever more and more urgent problems. The solution of such problems requires the inclusion of scientists-representatives of various branches of learning, as space medicine is a complex discipline, encompassing the aggregate of inter-related medico-biological, biophysical, physico-chemical, engineering-design and many other scientific studies directed toward creating optimum conditions for human life in outer space.

A definite contribution to the solution of urgent medico-biological problems of cosmonautics is being made by scientists of socialist countries cooperating in the "Interkosmos" program. As the result of many years of joint work, well-known successes have been achieved in carrying out a number of scientific and practical themes of space biology and medicine. Along with particular, analytical questions, more general theoretical problems have also been resolved. A harmonious combination of theoretical and practical studies will, in the final analysis, help develop recommendations regarding the creation of an optimum regime of work and rest for cosmonauts. /5

This book, "The Function of the Body and Factors of Space Flight," sums up the first five years of joint work in space biology and medicine by specialists of socialist countries.

The book consists of three sections (space physiology, radiation safety of space flights and pharmaco-chemical protection against ionizing radiation), summing up the basic works in the area of space biology and medicine carried out in the "Interkosmos" program.

One characteristic of this monograph is that it only presents the results of joint studies by scientists of socialist countries.

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*Translator's note: Numbers in margin indicate pagination of original foreign text.

There is no doubt that the present book will enrich space biology and medicine with new data and will help strengthen scientific contacts between scientists of socialist countries — specialists in the area of cosmonautics.

USSR Deputy Minister of Public Health — A. Burnazyan

TABLE OF CONTENTS

Annotation ii

Foreword iii

Cooperation between socialist countries in space biology and medicine within the framework of the "Interkosmos" program. N. N. Gurovskiy (USSR) 1

Space Physiology

The problem of hypoxia, hyperoxia and hypercapnia in space physiology. N. A. Agadzhanyan, P. M. Gramenitskiy, Ye.A. Kovalenko (USSR), I. Dvorzhak, M. Moravek (Czechoslovakia), L. Palash (Hungary) 20

The internal nature of functional disturbances arising in the body under the effect of extreme factors. P. M. Gramenitskiy, Ye. A. Kovalenko, (USSR), I. I. Dvorzhak, M. M. Moravek, Ya. I. Tsmiral (Czechoslovakia), K. Zlatarev, K. Kh. Kunchev (Bulgaria) 41

Internal thermotopography and shifts in general thermal balance in man under special heat transfer conditions. S. M. Gorodinskiy, P. M. Gramenitskiy, Ye.I. Kuznets, O. Ye. Ozerov, E. V. Yakovleva (USSR), P. Groza (Romania), S. Kozlovskiy, Yu. Naremski (Poland) 50

Tolerance of acute hypoxia while performing operator activity and after a prolonged period under altered gas environment conditions P. Bloshchinskiy, L. Golets (Poland), N. A. Agadzhanyan, A. V. Sergiyenko (USSR) 63

On characteristics of caloric nystagmus in healthy persons. D. Bodo (Hungary), V. P. Baranova, E. I. Matsnev, M. Ya. Yakovleva (USSR)... 74

Gas exchange and the coagulation system of the blood during the effect on the body of high concentrations of oxygen and carbon dioxide. L. Palosh (Hungary), N. A. Agadzhanyan, G. A. Davydov, B. K. Rybakov, A. S. Sergiyenko (USSR) 80

Correlation changes in EEG, conditioned and behavioral reactions with various degrees of oxygen insufficiency. N. A. Agadzhanyan, I. N. Zakharova, L. V. Kalyuzhnyy (USSR), I. Dvorzhak, M. Moravek, Ya. Tsmiral (Czechoslovakia) 100

Studies of hypokinesia in animals to solve urgent problems of space biology and medicine. S. Baran'ski, K. Bodya, V. Reklevska, L. Tomashevska (Poland), M. S. Gayevskaya, Ye. I. Il'ina-Kakuyeva, G. Katsyuba-Ustilko, Ye. A. Kovalenko, L. M. Kurkina, E. S. Mailyan, V. S. Nevstruyeva, V. S. Oganov, A. N. Potapov, V. V. Portugalov, B. M. Fedorov (USSR) 113

References 129

Space Radiobiology

Justification of permissible doses of radiation during prolonged space flights. Yu. G. Grigor'yev (USSR), Kh. Abel' (GDR), V. Varteres (Hungary), N. Nilolov (Bulgaria), Z. Karpfel', M. Prislichka (Czechoslovakia) 141

Study of a number of biochemical indices of the blood and tissue of dogs after prolonged γ-radiation. I. Alers, E. Alersova, T. Praslichka (Czechoslovakia), E. Mishurova, A. Sedlakova, Zh. Malatova (Czechoslovakia), A. A. Akhunov, B. A. Markelov (USSR) 145

A study of immunological reactions in dogs exposed to prolonged chronic radiation. I. V. Konstantinova, Yu. G. Grigor'yev, B. A. Markelov, A. S. Skryabin, V. M. Zemskov, I. S. Vasil'yev (USSR), Yu. K. Veysfeyler, I. Iokai (Hungary)..... 152

Radiobiological effects of heavy ions and protons. N. I. Ryzhov, S. V. Vorozhtsova, Ye. A. Krasavin, T. Ye. Mashinskaya, N. Ya. Savchenko, B. S. Fedorov, V. F. Khlanonina, V. N. Shelegedin (USSR), L. Gut, L. Sabo (Hungary), M. Minkova, I. Nikolov (Bulgaria)..... 163

The effect of synthetic homopolymer poly I:C on the synthesis of nucleic acids, protein and interferon in spleen cells normally and during radiation. Ye. N. Antropova, I. V. Konstantinova, B. B. Fuks (USSR), M. Ya. Talosh, Yu. K. Veysfeyler (Hungary)..... 174

Individual radiosensitivity and its diurnal variations. Yu. N. Druzhinin, Yu. G. Grigor'yev, G. N. Podluzhnaya (USSR), M. Pospishil (Czechoslovakia) 185

Daily rhythms of radiosensitivity of animals and several determining causes. Yu. P. Druzhinin, T. S. Malyutina, V. M. Seraya, G. P. Rodina (USSR), A. Vatsek, A. Rakova (Czechoslovakia) 193

The temporal organization of processes of cell reproduction and its connection with rhythms of radiosensitivity of the body. Yu. P. Druzhinin, Yu. A. Romanov (USSR), A. Vatsek (Czechoslovakia) 203

Development and creation of conditions for the radiation of biological objects with high-energy protons. V. P. Popov, A. I. Portman, M. A. Sychkov, A. V. Kolodkin (USSR), I. Nikl (Hungary) 216

Physical conditions for conducting radiobiological experiments in beams of accelerated particles with high linear energy transfer. Ye. I. Kudryashov, A. M. Marennyy, V. I. Popov (USSR), K. Aykhorn, G. Ertsgreber (GDR)..... 226

Isotope α-irradiators for radiobiological research. V. Drasher (Czechoslovakia), Ye. I. Dudryashov, O. M. Meshcheryakova, A. M. Marennyy (USSR) 236

Microdosimetry and radiation danger of cosmic rays. K. Gunter, V. Schultz (GDR), A. T. Gubin, Ye. Ye. Kovalev, V. A. Sakovich (USSR).. 247

Methodological questions of creating tissue-equivalent phantoms. A. V. Kolodin, V. I. Popov, M. A. Sychkov (USSR), I. Nikl, M. Erdei, O. Eyben (Hungary) 258

References 264

Pharmaco-chemical Protection Against Ionizing Radiation

Pathogenetic validation of the use of biological protective agents and means of early treatment in cases of radiation injury simulating radiation effects under space flight conditions. V. D. Rogozkin (USSR), V. Varteres, L. Sabo (Hungary), N. Groza (Romania), I. Nikolov (Bulgaria) 273

The biological effect of prolonged radiation and ways of selecting new anti-radiation drugs effective in this kind of radiation injury. V. D. Rogozkin, K. S. Chertkov (USSR), I. Nikolov (Bulgaria) 296

Experimental-clinical validation of the use of amitetravit, ATP and autologous bone marrow in radiation injuries caused by prolonged radiation. O. M. Atamanova, L. M. Vodyakova, N. I. Gvozdeva, S. A. Davydova, L. P. Ignasheva, V. D. Rogozkin, M. F. Sbitneva, L. M. Ostroumova, M. V. Tikhomirova, A. G. Fedotenkov (USSR), I. Nilolov (Bulgaria) 310

References 322

Conclusion 335

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COOPERATION BETWEEN SOCIALIST COUNTRIES IN SPACE BIOLOGY AND MEDICINE WITHIN THE FRAMEWORK OF THE "INTERKOSMOS" PROGRAM

/6

In 1965 socialist countries, expressing common interest in the most effective exploration and use of outer space for peaceful purposes, conducted negotiations in the Soviet Union on the possibility of cooperation in the area of science and technology. As a result of mutual scientific consultations and negotiations, in 1966 the following basic directions of such cooperation were determined: space physics, space communication, space meteorology, space biology and medicine.

To control and coordinate the works, in each of the cooperating socialist countries an appropriate national coordinating agency was created. A Council on International Cooperation in the exploration and use of outer space for peaceful purposes ("Interkosmos") was organized at the USSR Academy of Sciences. The specific mission of coordinating research in space biology and medicine /7 was entrusted to the USSR Ministry of Public Health.

A meeting of experts-representatives of socialist countries was conducted in April, 1967, in Moscow.

Upon the proposal of the Soviet Union, the experts from the socialist countries accepted the following scientific problems for cooperation in space biology and medicine:

– the effect on the body of extreme space flight factors (space physiology);

– radiation safety of space flights and search for pharmaco-chemical means of anti-radiation protection;

— medico-biological aspects of closed ecological systems.

For realization of the program, specialists of socialist countries which participated in the meeting worked out drafts of bi- and multilateral protocols (agreements) on specific scientific-research subjects, subject to discussion and approval by the national agencies of each cooperating country party.

The drafts of agreements specified the following basic forms of cooperation:

— reciprocal visits of specialists to carry out joint scientific work and become acquainted with scientific research;

— reciprocal visits of scientists and specialists to give lectures and conduct consultations;

— joint scientific conferences, symposiums and meetings;

— exchange of scientific data, methods and instruments, technical documents and other materials necessary for joint research.

Coordinated programs of cooperation are controlled through the national coordinating agencies. This function is carried out within the country by permanent working groups. The latter shall also control the scientific-methodological level of works and search for measures to improve forms of cooperation.

The extremely broad range of questions encompassed by the program of cooperation necessitates inclusion of a large number of scientific-research institutions, clinics and laboratories of socialist countries. In determining the specific participants in joint works, the traditional scientific orientation of the research institutions, their operating equipment and the presence

of qualified personnel were taken into consideration.

The leading institution in the USSR in carrying out joint works on the part of Soviet cooperative obligations is the Institute of Medico-biological Problems of the USSR Ministry of Public Health, organizing joint research in space physiology and radiation safety. Joint efforts in the search for anti-radiation drugs are being carried out by the Institute of Biophysics of the USSR Ministry of Public Health. The scientific bases of a number of other research institutes of the country are also being used for the studies.

In accordance with the approved proposition for summarizing cooperation, discussing results of joint research, correcting subjects and solving other organizational questions of cooperation, annual meetings of the permanent combined working group are held by turns in each country. /8

Meetings of the working group of socialist countries on space biology and medicine were conducted in July 1968 in the Soviet Union, in September 1969 in the People's Republic of Bulgaria, in September 1970 in the Hungarian People's Republic, in September 1971 in the Czechoslovakian Socialist Republic, in June 1972 in the Polish People's Republic and in June 1973 in the German Democratic Republic. The regular meeting of the working group will be held in 1974 in the Socialist Republic of Rumania.

According to a mutual agreement, at the same time the working group is meeting, a symposium on subjects of joint research and several urgent problems of space biology and medicine will be conducted. In addition, new scientific reports on various questions of space biology and medicine of general interest to cooperating countries are usually made at the symposium. For example, at the symposium in Dubno in 1968, a report was made on urgent questions of radiation safety of space flights, giving data on the radiation situation and results of radiobiological observations during the flight of the manned "Vostok" and "Soyuz" craft. At the symposium in Varna (People's Republic of Bulgaria) data were presented on the state of several physiological functions of cosmo-

nauts as the result of flights in "Soyuz-4" and "Soyuz-5" craft and physiological-hygienic data on a year-long medical and technical experiment. The symposium in Prague (Czechoslovakian Socialist Republic, 1971) presented several results of medical and biological studies conducted during the flight of "Soyuz" spacecraft. At the symposium in Warsaw (Polish People's Republic, 1972) a very interesting report was heard concerning certain medical studies during the flight of the "Soyuz-Salyut" space station.

At meetings of the working group, work in corresponding directions of research is summarized from plenary sessions and sectional meetings; works on specific subjects for the past period are summarized, compliance of research with approved protocols (agreements) and work plans is determined, plans are outlined for future research on a particular subject, etc. All this is drawn up by corresponding protocol.

Let us dwell on several reviews of work conducted by socialist countries on space physiology, space radiobiology and in the search for anti-radiation drugs.

Studies in space physiology are one of the basic directions of cooperation between specialists of socialist countries in the "Interkosmos" medico-biological program.

Space physiology, as space medicine as a whole, is intended to serve two basic practical purposes: preserving the health and maintaining a high level of working capacity of cosmonauts during the flight, as well as ensuring their painless transition to ordinary living conditions after return to Earth from a prolonged space flight. To achieve such purposes, space physiology studies the effect on the body of various specific space flight factors and the entire set of conditions in which man finds himself in carrying out space expeditions; it investigates the function of the body under such conditions, determines the limits of permissible variations in such factors and substantiates prophylactic measures to prevent or weaken their undesirable effect. /9

Along with solution of practical problems, space physiology is called on to study unusual factors and conditions of space flight to strengthen the theoretic base of space medicine.

Because of the breadth and complexity of the problems of space physiology, its close contact with a number of other biological and medical disciplines is necessary — with general and pathological physiology, aviation medicine, occupational hygiene, many clinical specialties, etc. Individual questions necessary for solution of space physiology problems can be successfully worked out in the sphere of these related disciplines, which significantly expands the opportunity for cooperative work between specialists of different countries on the physiological level of works conducted in the "Interkosmos" program.

The physiological orientation of works conducted in the "Interkosmos" program, of course, includes study of the most urgent problems of space physiology. Of these, first place is occupied by the problem of weightlessness. Indeed, exclusion from human living conditions of the force of gravity — a factor accompanying the entire evolution of life on Earth — must, undoubtedly, have an important effect on the course of physiological processes.

The difficulty of studying the problem of weightlessness is that this condition is now impossible to simulate completely on Earth. However, literature data and experimental research conducted in the Soviet Union and the United States indicate that an approximate model of weightlessness (according to a number of indices) can be produced by a state of hypodynamia or immersion in water. Hypodynamia in a horizontal position of the body (staying in bed) can, with certain tolerance, be considered a model reproducing in the body a number of symptoms typical of weightlessness. At the same time, this state also has independent significance as an integral condition of prolonged space flights and as a factor acquiring general biological and social meaning in the modern era of scientific and technical progress and wide use of transportation facilities.

Therefore, in physiological works of the "Interkosmos" program, the problem of hypodynamia has been given due attention. Specialists from Poland, the USSR and Czechoslovakia, in comprehensive studies on animals, investigated various effects of hypodynamia. Both the general condition and functional capacity of the body and individual physiological functions were evaluated, particularly motor activity, neuro-muscular apparatus, gas-energy metabolism, water-salt and especially calcium exchange, the neuro-hormonal regulator system, histochemical and morphological indices of various structures of the body. The results have important scientific and practical significance, first of all, for evaluating consequences of the inevitable limitation of motor activity during space flight and, secondly, for analyzing the effect of weightlessness on the body. These data give bases for recommendations directed toward prevention of the undesirable effect of hypodynamia and weightlessness on the body. /10

The opposite of weightlessness is the problem of overload, study of which in countries cooperating within the framework of the "Interkosmos" program has been placed on a sufficiently precise technical base and necessary scientific level. In space physiology overloads emerge as a significant factor in active portions of space flight; specific conditions of this factor are the rapid transition from it to weightlessness in the initial stages of the flight and, what is more important — the effect of overloads on the body after prolonged weightlessness at the end of the flight. This specific requires a new approach to consideration of this problem for more accurate definition of seemingly already justified and established standards and criteria of overload tolerance.

Specialists of Bulgaria, Poland, the USSR and Czechoslovakia, studying humans, obtained comprehensive data on tolerance of overloads with various characteristics; the degree of tolerance was compared with resistance of the organism to certain other space flight factors. Several means of raising human resistance to overloads were also justified (the preliminary effect of an altered gas medium, in particular, prolonged adaptation to hypoxia in a pressure chamber or in a high mountain climate, the use of helium-oxygen mixtures during overloads, etc.).

An important place in plans for physiological studies is occupied by the problem of vestibular stability and maintaining spatial orientation during rocking and Coriolis accelerations. In this area, specialists of socialist countries made systematic tests of humans, studying various symptoms, particularly hormonal indices of vestibular stress. It was found that disturbances of spatial orientation after the effect of Coriolis accelerations and hypokinesia are determined not only by specific changes in receptor function but also by hemodynamic shifts. These data help determine the proper approach to developing measures directed toward increasing vestibular stability, particularly the use of drugs for this purpose. Several drugs were tested in the course of joint studies conducted by Bulgarian and USSR specialists. These studies produced characteristics of caloric nystagmus in healthy persons, making it possible to recommend the use of this test in occupational selection of cosmonaut candidates.

A very important place in plans of physiological studies is given to the effect on the body of factors of the gaseous medium. Specialists of practically all cooperating countries actively participated in development of its various aspects. These studies produced data characterizing the physiological effect on the body of various factors of the gaseous medium and, what is even more important, extensive material for justifying a rational spacecraft cabin atmosphere, taking into account the possible future use of various indifferent dilutant gases. At the same time, the possibility was shown of using basic vital factors of the gaseous medium for forming a dynamic atmosphere having a general toning effect on the body during weightlessness and hypodynamia; measures for preventing decompression disturbances and resultant requirements for a gaseous medium in the spacecraft cabin were justified. Finally, analysis was made of the critical effects of factors of the gaseous medium in relation to accident situations. /11

Thanks to active cooperation between specialists of socialist countries, the problem of the effect on the body of unusual temperature conditions was developed. These works studied the characteristics of heat regulating reactions during thermal effects and the temperature regime of the brain of ani-

mals under ordinary and altered conditions, deep physiological characteristics of the effect on the human body of a heated microclimate were obtained and the most informative criteria of extreme heat conditions were determined.

Studies of the effect of factors of the gaseous medium on the body, therefore, expanded ideas of the mechanism of their physiological action and made it possible to define accurately a number of important practical matters concerning formation of a spacecraft cabin atmosphere and prevention of overheating of people in a warm microclimate.

Nutrition and the creation of automatic life support systems for spacecraft crews were investigated by specialists of Poland, the USSR, Rumania and Czechoslovakia in a study of the digestive function during the effect of various space flight factors on the body, particularly hypokinesia, and in studies directed toward developing closed ecological systems. The characteristics of several single-cell algae as possible links in this system were ascertained; the biological value of individual food components derived from lower and higher plants was studied as well as the resistance of the body to space flight factors when food products obtained from biological components were included in the diet. Such works within the framework of "Interkosmos" are in the initial stage of development and formation.

Specialists of Bulgaria, Poland, the USSR and Czechoslovakia studied the psychological security of man in extreme conditions, using psychological, psychophysiological and electrophysiological methods, directed toward analyzing and characterizing the psychological state of man in stress situations. Results are of theoretical interest and can be used in selecting cosmonauts and predicting individual tolerance of extreme effects.

The above indicates that the physiological orientation of works within the framework of "Interkosmos" is developing successfully and is producing important theoretical and practical results. The expansion of the front of physiological research is also indicated by an ever increasing number of re-

ports and publications at regular symposiums in the "Interkosmos" program. As the program of the mastery of outer space expands and the length and distance of manned space flights increase, ever greater importance is also acquired by the problem of the radiation safety of such flights, protection of the crew and biological links in the life support system against cosmic ionizing radiation.

According to physical characteristics, spatial distribution and also /12
taking into account the conditions of each specific flight, especially dangerous with regard to radiation are galactic cosmic rays (GCR), the radiation belts of the Earth (RBE) and solar flare radiation (SFR). At the present time, in evaluating radiation danger we must also take into consideration such sources of radiation as on-board isotope units, nuclear engines and nuclear-energy units. The radiation of cosmic rays becomes especially evident in prolonged space flights. Calculations show that the yearly dose of radiation from cosmic rays can reach 100 rem. The presence in the cosmic ray composition of heavy ions, having a great ionizing capacity, presents radiobiologists with a number of new problems.

One interesting radiation effect is the observation by cosmonauts during flights to the Moon of light flashes or lines with a frequency of 1-2 per min. In accelerator tests it was shown that heavy ions are able to cause light flashes or lines when they intersect the human retina, adapted to dark. At the present time it can be assumed that "flashes" and "lines" can be perceived if the energy loss is high — approximately 10 kev/μμ. Therefore, the majority of fast and slow particles of cosmic radiation, leading off with nitrogen, can cause the effect of luminescence to cosmonauts.

In connection with the above, results of radiological studies with heavy ions are becoming especially important for evaluating the radiation danger of space flights.

Radiobiological studies with particles with high linear losses of energy were preceeded by a great deal of work in creating the physical conditions for radiation and studying depth dependence of an absorbed dose and the energy spectrum of accelerated particles in tissue-equivalent materials. These works were carried out by GDR and USSR physicists at cyclotrons in Dubno and Rossendorf.

Radiobiologists of Bulgaria, Germany and the USSR made a large number of experiments with heavy ions on viruses, bacteria and phages. Tests were made with ions of boron, carbohydrate, neon and argon. The effects of deactivation and mutagenesis were studied, the dependence of the cross section of deactivation of bacteria on particles with linear losses of energy was shown as well as the possibility of modifying the radiobiological effect of heavy ions.

Additional opportunities to study the effect of rays with various linear loss of energy are opened up by the use of new methods of α-radiation of biological objects. The result of the joint efforts of USSR and Czechoslovakian specialists was the creation of an α-irradiator. We must note the great carefulness in evaluating the physical field of radiation and the distribution of the absorbed dose in the biological object.

A special problem is evaluating the dose when a body is irradiated with heavy ions. Expression of the dose in rads, as is present accepted practice, presupposes macroscopic homogeneous radiation, which, in fact, is not the case with heavy ions. Therefore, physicists of the GDR and the USSR produced a mathematical description of the possible microdistribution of absorbed doses, the problem of microdosimetry and the role of this phenomenon in the formation of radiation injury.

Proton radiation from solar flares is a potential danger for any kind of /13
space flight. In evaluating the radiation danger of solar flares, it is important to evaluate the distribution of absorbed doses in the body of the cosmonaut, as well as the amount of radiation of critical organs in radiation of the body with protons of various energy. Calculations show that differences

between absorbed doses can be significant. An analogous situation occurs in evaluating the danger of Van Allen radiation belts. In addition, ideas of the formation of the absorbed dose in the body of a cosmonaut after protection, taking into account the amount and energy spectrum of secondary radiation, must be based on direct dosimetric measurements in a dummy under actual space flight conditions.

Hungarian and USSR specialists conducted a great deal of joint work in creating a tissue-equivalent dummy. The optimum composition of the dummy was determined to evaluate the interaction between tissue and protons, neutrons and γ-radiation. Culmination of the studies was the creation of an experimental model of a human dummy with movable joints.

An extensive program of radiobiological experiments with high energy protons carried out in the Dubno accelerator was preceeded by work of Hungarian and USSR physicists creating conditions for radiation, characterizing dose fields and absorbed doses in radiated objects and monitoring.

A team of radiobiologists from Bulgaria, Hungary and the USSR conducted research to study the characteristics of the biological effect of protons of various energy from 50 to 660 Mev. Both immediate and remote effects were evaluated, the effect of protons on slowly and rapidly regenerating tissues and the possibility of restoring disturbed functions were studied and the radiosensitivity of lymphocytes in man were tested in radiation with protons in various phases of the cellular cycle. Analysis of material obtained in radiation of experimental animals, cells of mammals in culture and bacteria gives bases for assuming that the relative biological effectiveness of protons with energy of 50-60 Mev is equal to one or differs insignificantly from such. However, further reduction of energy shows a tendency towared a slight increase in RBE. RBE coefficients above one were obtained in radiation of the cornea of mice with protons with energy of 10 Mev. Also extremely interesting are data on the effect of proton radiation on the induction of interferon.

In establishing standard levels of radiation for prolonged space flights, it is very important to have available reliable information on the possible development of radiation effects during chronic radiation of the body in simulation of a dose level and rate close to actual flight conditions. In this respect the "chronic experiment" organized in the USSR in 1967 becomes very important. In this experiment 240 dogs were exposed for 3 and 6 years, either to chronic daily radiation only or to joint radiation (chronic and acute) in various total doses (21, 63, 125 and 190 rad per year).

An important addition to the "chronic experiment" data is material from studies of the effect of prolonged radiation on the population of animal cells outside the organism, as well as examination of possible mechanisms of the reaction of the body to chronic radiation. /14

There is as yet insufficient information on the modifying influence of space flight factors on the radio biological effect. It is well known that the human body reacts to the least changes in the environment, especially those it rarely encounters on earth. The volume of knowledge on the combined effect of space flight factors is limited and does not at the present time make it possible to formulate a comprehensive hypothesis regarding possible consequences of the interaction between radiation effects and factors of a different nature during prolonged space flight. Synergism of the effects of radiation and other physical factors is very important in evaluating the danger of space flight. Factors of a non-radiation nature can probably change the radiosensitivity of the body. The effect of space flight factors can also be considered as a case of the modifying effect of biological factors on radiation injury.

Works in this direction conducted in socialist countries deserve every encouragement.

Extremely significant are reports of the range of changes in individual radiosensitivity under various conditions. In this respect, noteworthy are the

works conducted in the "Interkosmos" program dealing with the temporal organization of cell reproduction processes and its connection with radiosensitivity rhythms of the body; daily variations in radio sensitivity; finding causes and factors determining daily rhythms of radiosensitivity. These works were carried out jointly by specialists of Czechoslovakia and the USSR.

Other important problems are the prediction of radiosensitivity and purposeful selection of radioresistant and radiosensitive organisms for space flight. A number of works in this direction have been carried out by Czechoslovakian radiobiologists.

A complicated problem in ensuring the radiation safety of space flights is organizing dosimetric service on board the spacecraft. In this respect, valuable material was obtained by specialists of Hungary and the USSR in studying dosimetric characteristics of thermoluminescent detectors. It was shown that thermoluminescent detectors made of aluminophosphoric glass (APG) can be used successfully to measure integral doses of cosmic radiation. They are resistant to various space flight factors and chemically inert; they preserve dose information well during prolonged storage under high temperature conditions. The sensitivity of APG detectors is not less than Li or F detectors; they can measure integral doses in the range between 10^2 and 10^4 R. APG detectors are practically transparent to self-radiation and do not change transparency during radiation in doses at least to 5000 R.

A measurement device has been perfected for characterizing doses accumulated by thermoluminescent dosimeters.

Summarizing all the above, we feel it necessary to indicate that today special attention must be given to the biological effect of heavy ions on the body of cosmonauts. Methods of proper evaluation of such danger are not yet ascertained. Only expansion of radiobiological research in space and experiments in heavy ion accelerators will solve this problem. /15

Also requiring careful evaluation are the effects of the combined action of ionizing radiation and other space flight factors. It seems important to obtain quantitative data on coefficients of residual radiation injury and the rate of recovery under chronic and prolonged radiation conditions.

Experience shows that successful solution of very complex problems of space radiobiology is possible by combining the efforts of radiobiologists and physicists, including scientists from different countries using different methods of research and obtaining comprehensive scientific information in experiments.

An important place in the works of "Interkosmos" has been assigned to studies of "pharmaco-chemical protection against ionizing radiation." This is because, even with sufficiently powerful physical protection, especially in prolonged space flights, it has not been feasible to create conditions for the complete exclusion of accidents and radiation injuries to the crews of spacecraft, primarily from solar flares and cosmic rays.

Studies of drug protection against the effect of ionizing radiation by scientists of socialist countries in the "Interkosmos" program were made in three basic directions:

— the effectiveness of known means of chemical prophylaxis of radiation injuries was evaluated;

— means of biological protection able to raise natural radioresistance were worked out;

— the effect of early pathogenetic treatment of radiation sickness was studied.

In addition, special pharmacological studies were conducted to find the effect of anti-radiation drugs, their tolerance by animals and man during

various space flight factors.

On the basis of all this information, their correspondence was determined to technical requirements for the use of drugs during space flights.

In the first direction, important data was obtained as the result of joint studies by scientists of Bulgaria and the USSR. It was shown that such well-known radio-protectors as betamercaptoethylamine (mercamine), betamercaptopropylamine (propamine), disulfide betamercaptoethylamine (cystamine), 5-methoxytryptamine (mexamine), S-β-aminoethylisothiurea (AET), etc., show no positive effect during prolonged low-power radiation (1 R/min or less) simulating the effect of solar flares.

A connection was established between the effectiveness of chemical radio protectors and the power of radiation. Thus, with γ-radiation of 100 R/min or more, the anti-radiation effect of mercamine, propamine, cystamine and mexamine, administered parenterally for 5-15 minutes before radiation, was seen in a 50-70% increase in survival of the animals. With a dose rate of 10 R/min, the effectiveness of such chemical radio-protectors was seen in a 20-25% increase of survival. With reduction of exposure rate to 3.7 R/min the prophylactic effect of radio-protectors was decreased even more. Survival of the animals under such conditions was 13-17% greater than in the controls. No radio protection from mercamine, propamine, cystamine or mexamine was observed when the animals were radiated at 1 R/min or less. /16

It was also shown that multiple administration of chemical radio-protectors of the above listed groups can even increase the severity of the course of radiation sickness.

Data of Bulgarian and USSR scientists, obtained in tests on small laboratory animals, were verified by the results of cell culture studies. With prolonged radiation, the prophylactic application of mercamine had a negative effect, increasing cell death. As is known, after the introduction of mercamine

to animals its concentration in tissues decreases comparatively rapidly, which is often considered one of the causes of the reduced radio protective effect of this chemical radio-protector during prolonged radiation. Attempts to lengthen the protective effect of mercamine or increase it by retaining it in the tissues increased animal death. This becomes more understandable if we take into consideration the dependence of this cytotoxic effect of mercamine on both its concentration and the time of its contact with a cell.

Works of Bulgarian and USSR scientists in evaluating and analyzing the effectiveness of known chemical radio-protectors during prolonged radiation contraindicates their use during prolonged radiation, simulating the effect of solar flares.

Scientists of socialist countries, working on the problem of "Pharmacochemical Protection against Ionizing Radiation" concluded it is necessary to search for new paths of preventing radiation injuries which could arise under space flight conditions. Special attention has been given to developing anti-radiation drugs capable of preventing primary damage to genetic structures by ionizing radiation or activating post-radiation recovery in the initial stages of the pathological process. Basic works dealt with a search for drugs favoring repression of DNA by protein and for compounds activating in the cell enzyme systems restoring unique genetic structures. In this connection, in the "Interkosmos" program scientists of socialist countries have conducted numerous studies demonstrating the prospects for use in corresponding radiation injuries of biological protection capable of activating the synthesis of proteins and the adenyl-cyclase system, at the same time raising the general resistance of the body and immunological reactivity and the body's natural radioresistance as well as preparations which can modify the course of energy metabolism in cells before and after radiation. Subjected to the most thorough study were adenosine triphosphoric acid and amitetravit, which is a combination of amino acids (histidine and tryptophan) and vitamins (B_1, B_6, C and P), as well as anabolic steroids and polysaccharides. Works of Bulgarian and USSR scientists established a pronounced anti-radiation effect of ATP and

amitetravit in various kinds of radiation injuries simulating various kinds of radiation effects during space flight, i.e. in acute single, fractionated and prolonged radiation. /17

It was shown that ATP can prevent damage primarily in radiosensitive organs and tissues, reduce the destructive effect of ionizing radiation on bone marrow and the spleen and promote faster restoration of hemopoeisis.

Rumanian scientists revealed the ability of ATP to prevent functional damage to stomach secretion in irradiated animals and expressed opinions on the possible mechanisms of the anti-radiation effect of ATP on the intestinal syndrome in radiation injuries.

To determine prospects for the possible practical use of ATP and amitetravit as antiradiation drugs, especially noteworthy are the studies of USSR scientists on their effectiveness on dogs during prolonged radiation.

There is important evidence of the high anti-radiation effect of the protective-therapeutic administration of ATP in combination with a preparation of amitetravit in cases of severe radiation injury caused by prolonged radiation.

Thus, the data obtained by scientists of Bulgaria, Rumania and the USSR for the first time provided scientific justification of the theoretic possibility of producing an anti-radiation effect from drug prophylaxis of medical injuries on radiation models adequate for those which can arise during space flight, primarily during prolonged radiation simulating the possible radiation effect of solar flares.

Studies conducted in the "Interkosmos" program by scientists of Bulgaria, Rumania, the USSR and Czechoslovakia showed prospects for further investigative works regarding new anti-radiation drugs and formulas in this direction. Deserving special attention is the combined use of biological protection and other prophylactic and therapeutic drugs (derivatives of pyrimidine, phyto-

urease, ATP, etc.).

Taking into account the problems facing space medicine, it is also noteworthy that these anti-radiation preparations have no toxic side effects on the human body and have a wide range of therapeutic effect and effectiveness in various kinds of radiation injuries. All this convinces us of the feasibility of further experiments with these drugs to define more accurately indications and contraindications for their prescription and for working out recommendations for their use under space flight conditions.

The above material justifying the use of biological protection in space flights is also essentially supported and supplemented by a number of other studies conducted by scientists of socialist countries. In this respect we must note works studying the anti-radiation effectiveness of anabolic steroids and other hormonal preparations, as well as polysaccharides and endotoxins. As the result of joint research by scientists of Poland and the USSR, convincing material has been presented on the feasibility of using anabolic steroids in brief and prolonged radiation as a means of increasing natural radioresistance and favorably affecting the course of a number of biochemical processes and changes in the hematopoietic system. There are promising studies by scientists / 18 of Hungary and the USSR in relation to the use of polysaccharides of bacterial and plant origin to prevent and treat radiation injuries. Their ability to accelerate processes of post-radiation recovery in the blood system and increase survival of radiated animals has been established.

Hungarian and USSR scientists have shown that polysaccharides and endotoxins of bacterial origin can be used to cause a controlled effect on mother cells promoting differentiation processes in stem cells.

Interesting information for space medicine was obtained by scientists from Hungary and the USSR in relation to the ability of several polysaccharides of bacterial origin, besides their positive effect on the blood system during radiation with γ-rays and protons, to increase the amount of properdine and

interferon in the blood of radiated animals and change other indices characterizing increased immunological reactivity.

Important studies in the treatment of radiation injuries have been conducted by USSR and Czechoslovakian scientists on the effectiveness of the method of autotransplantation of bone marrow, the use of high polymer DNA and a number of antihemorrhagic drugs.

Czechoslovakian scientists have presented material indicating the feasibility of further study of the effect of high polymer DNA as a means of early pathogenetic treatment. As the result of joint studies by scientists of Czechoslovakia and the USSR, interesting material has been obtained justifying the use of a number of new anti-hemorrhagic drugs to treat radiation injuries (ellagic acid, serotonin, glutamine, etc.).

Studies in the "Interkosmos" program, conducted to develop pharmacochemical protection against ionizing radiation, and in other areas of cooperation between scientists of socialist countries, have, therefore, made it possible to produce a large amount of varied and valuable material, creating a basis for the purposeful development of recommendations for the use of drug prophylaxis and treatment of radiation injuries which can develop during space flights.

Experiments in space biology and medicine, conducted jointly by specialists of socialist countries in the "Interkosmos" program, are very important for mutual understanding and strengthening cooperation between these countries and are of significant use in the exploration and use of outer space for peaceful purposes.

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SPACE PHYSIOLOGY

/19

The Problem of Hypoxia, Hyperoxia and Hypercapnia in Space Physiology

One of the basic factors affecting the human body in pressurized compartments is the surrounding air medium whose composition is largely responsible for its general condition and working capacity.

As the time man spends in pressurized cabins lengthens, requirements for a gas medium will become increasingly strict and must in an ever greater degree take into consideration not only the direct effects of the medium on the body, but also the reverse influence of the body on the gas medium surrounding it. Interests in finding broader technical and biological resources for supporting human life in space have necessitated investigation by specialists of socialist countries of different variants of an atmosphere, particularly physiological grounds for permissible concentrations of oxygen, carbon dioxide and other gases.

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In the complex influence of various factors of an altered gas medium on the body, the greatest danger is presented by acute insufficiency of oxygen.

Hypoxia and its effect on the body. Although hypoxia has long attracted the attention of various kinds of researchers, many aspects of the problem have not yet received sufficient study. We still do not have available complete data revealing the intimate mechanisms underlying adaptation to oxygen insufficiency. In broadest form, two types of adaptative reactions of the body to hypoxia are considered — extracellular compensatory reactions (respiratory, hemodynamic, humoral), providing a normal oxygen supply to cells, and tissue-cellular accomodative reactions, responsible for metabolism in accordance with

the oxygen supply.

Adaptation reactions of the first type have been sufficiently well investigated.

Along with extracellular compensatory reactions, oxidation enzymes of tissues enter the fight for oxygen. It is known that oxygen molecules in tissues follow a complicated path until, colliding with an electron stream, they acquire the ability to combine with ionized hydrogen, forming water as the end product of biological oxidation. It has been shown that the latter process occurs in mitochondria — cellular organelles whose morphological structure and physiological function is presently under comprehensive study. Until then, all prior oxygen compounds are temporary, having transport significance and easily decomposing under certain conditions.

In the opinion of N. A. Arkahngel'skaya (1946), accomodative changes to hypoxia occur in the cell itself, probably only in the nuclear apparatus, regulating cellular metabolism [13].

According to the data of Kamakura, hypoxia primarily affects membrane permeability [106]. The use of certain drugs (ethylcarbamate) prevents reduction of the calcium content in human erythrocytes and increase in the sodium content. Ethylcarbamate in subnarcotic doses markedly lengthened the survival time of rats at an altitude of 12,000 m and doubled that of an isolated frog heart in a nitrogen atmosphere.

V. N. Chernigovskiy correctly indicates that "the cell would not be able to be a cell if at some moment it is not surrounded by an extremely thin membrane with selective permeability" [91].

To understand the physiological mechanisms of hypoxic disturbances and work out preventive measures directed toward ensuring the safety of high altitude flights, it is of interest to study the characteristics of gas exchange

in extremely rarefied atmospheres. The basic condition of normal oxygen supply of the body, in fact, comes down to maintaining sufficient pO_2 in alveolar air, which is the true gas medium for the living organism. It has been shown that with reduced atmospheric pressure, pO_2 in alveolar air drops more slowly than in the surrounding air. The extremely interesting fact that by itself time spent at the same altitude causes a regular reduction of pO_2 and pCO_2 has been established in experiment. The respiratory coefficient also changes. Thus, / 21 during an hour spent at an altitude of 7500 m, the oxygen content in alveolar air is reduced from 40 to 30 mm. Hg [5]. Oxygen saturation of the blood in subjects at this altitude fluctuates widely (from 55 to 76%).

In physiology, external gaseous exchange is comparatively well studied. However, the literature contains extremely inadequate information on the dynamics of gases directly in tissues of a living organism. Of course, study of the dynamics of oxygen in tissues of vital organs (brain, heart) is of paramount importance, especially in studying the effect of such factors of space flight as hypoxia, hyperoxia and hypercapnia.

Back in 1922 the Czechoslovakian scientist, Nobel Prize laureate Ya. Geyrovskiy, proposed a new electrochemical method of conducting qualitative and quantitative analysis of various substances, including oxygen, in body fluids. This method has received world recognition and wide distribution in various areas of chemistry, technology and biology. In the past ten years the use of numerous variants of the polarographic method has also begun in physiology. Noteworthy are the studies of Serák [122], Vacek and Sevik [127], which proposed an original design for a unit to determine tissue respiration and electrodes to determine oxygen tension in the blood and tissues.

Regular study of the dynamics of oxygen tension by the polarographic method directly in tissues of the living organism during the effect of several aviation and space flight factors has also begun in the USSR [44, 46, 47, 75]. As is known, the basic etiological factor in the development of hypoxia is the level of oxygen tension directly in tissues, primarily in brain tissue

most sensitive to oxygen insufficiency. Therefore, in animal tests a detailed study was made of pO_2 in the cortex and subcortex (hypothalamus) of the brain during ascent in a pressure chamber to various altitudes. It was shown that in the initial stage breathing air pO_2 in brain tissues of dogs and rats is 39±1.3 and 32±1.2 mm. Hg. In such cases unique rhythmic fluctions are observed in the pO_2 level which, evidently, reflect the existence of a self-oscillating process of oxygen supply and consumption. In ascent to altitudes of 4, 6 and 8 km, pO_2 in brain tissues was reduced in dogs to 22, 16 and 10 mm. Hg respectively and in rats at altitudes of 2, 4, 6 and 8 km fell to 25±0.8, 17±0.6, 13±1 and 7±0.5 mm. Hg respectively. At the same time, a change also occurred in basic physiological functions in the form of an increase in pulse and respiration rates, EKG changes, as well as intensification of bioelectric activity of the brain on the EEG with gradual predominance of slower biopotential rhythms. Further ascent to altitudes of 10 and 12 km and remaining at an altitude of 12 km for 2 minutes caused a drop of pO_2 in brain tissues of dogs to 4.3±1.4 mm. Hg and to zero respectively. In rats pO_2 of the brain at these altitudes dropped to 4±0.7 and 1±0.2 mm. Hg [75].

In the light of modern ideas of biological oxidation processes, it must be considered that a pO_2 level less than 4-5 mm. Hg can be critical, as under such conditions of oxygen tension the rate of its consumption in mitochondria begins to drop [97]. A special study of this question, conducted on mitochondria extracted from brain tissues, showed that a pO_2 level under 5-4 mm. Hg actually causes the rate of oxygen consumption to slow down [45]. It is interesting /22
that at an altitude of 9-10 km, when pO_2 in brain tissues reached critical levels, there were simultaneous sharp hypoxic disturbances in the form of excitation, convulsions, changes in EEG rhythms, etc. [75]. Therefore, reduction of pO_2 in brain tissues to levels critical for mitochondria also causes a chain of acute hypoxic disturbances. This important fact reveals a possible path for searches for protection against hypoxia either in the form of increasing the pO_2 level or by means of reducing the critical level of oxygen tension during the effect of certain protective means against hypoxia.

In addition, it has been established that during rapid ascent to altitude of 12 km, pO_2 in arterial blood is reduced more rapidly and to a greater degree than in venous blood of the jugular vein. This causes the arterial-venous difference first to decrease and at an altitude of about 10 km to disappear completely. What is more, with rapid ascent to altitudes above 9-10 km, pO_2 of arterial blood falls even lower than that of venous blood. This fact indicates that beginning with these altitudes, there is a qualitatively new process of gaseous exchange between the body and the atmosphere: there is not an intake but a washing out of the body of already minimal reserves of oxygen.

Numerous studies have shown that in breathing atmospheric air at critical altitudes (above 10,000 m), as in breathing pure oxygen at altitudes above 15,000 m, the reserve time in man is approximately the same, 15-30 seconds. This becomes understandable if it is taken into consideration that in approximately this time oxygen circulating in the blood reaches the heart and brain from the lungs. Such a fast rate of acute hypoxia at high altitudes is due not only to the reduction of partial pressure of oxygen in inspired air, but also to symptoms of degasification (deoxygenation) of the body, typical of such conditions. With extreme degrees of rarefaction of the atmosphere, the body loses from the blood through the lungs even those minimal amounts of oxygen which are in body tissues and hemoglobin, dissolved in tissue mediums and especially in the blood in the same way as it is lost from blood outside the body under conditions close to a complete vacuum. Degasification of the body is already observed at altitudes over 10,000 m and becomes especially pronounced with total pressure of 90 mm. Hg (altitude 15,000 m) where alveolar pO_2 approaches zero, although physically there is still oxygen in the atmosphere. Based on this, some authors consider an altitude of 15,000 m with regard to the oxygen supply as an altitude equivalent to conditions of free space [125].

For aviation and cosmonautics, of important scientific and practical significance is finding the dependence of the dynamics of basic functional systems of the body and behavioral reactions on the oxygen regime of the body.

It is known that adaptation to hypoxia is a very complex and multifaceted process involving practically all organs and systems of the body. However, the resulting function characterizing the effectiveness of compensatory-adaptational reactions of the body is the level of oxygen saturation of the blood, expressing the ratio between the amount of bound oxygen and the oxygen capacity of the blood.

The degree of oxygen saturation of arterial blood is largely responsible for oxygen tension in tissues, meaning also the normal course of oxidation processes. In turn, normal arterial oxygen saturation requires a certain correlation between air exchange — alveolar ventilation (AV) and pulmonary circulation (PC) — as the degree of oxygen saturation of the blood depends primarily on the AV/PC ratio, the rate of diffusion of oxygen and the functional state of the cardio-vascular system of the body as a whole. /23

In tests on humans with the effect of acute hypoxia (breathing pure nitrogen or helium) a certain correlation was established between oxygen saturation of the blood, EEG changes and behavioral reactions [6]. Disturbances of higher nervous activity developed in man under such conditions with 75-80% oxygen saturation of the blood. With 65% oxygen saturation of the blood delta waves predominate on the EEG and high-frequency fluctuations in biopotentials practically disappear; errors appear in performing simple arithmetic problems, symptoms of perseveration are observed. This indicates the presence under such conditions of a functional dissociation between the cortex and subcortical areas. As hypoxia increases further, with oxygen saturation of the blood below 65%, as a rule, loss of consciousness occurs.

Interesting data have been obtained on fighter pilots and students in studying the effect of acute hypoxia, corresponding to an altitude of 7500 m, on operator activity [18]. It has been established that in performing complex coordinated operator activities in a trainer during acute hypoxia, reaction time lengthened significantly and the number of errors increased. After 5-6 minutes at an "altitude" of 7500 m, oxygen saturation of the blood decreased 70%; along

with serious deterioration of working capacity, menacing symptoms appear (convulsive twitching of the operating hand, and then loss of consciousness).

A study of several neurochemical mechanisms of brain reactions showed that under conditions of increasing oxygen insufficiency, an initial predominance of cholinergic excitation is observed in anterior-lateral sections of the hypothalamus and at altitudes above 5000 m — adrenergic excitation of posterior-medial sections of the hypothalamus and the reticular formation of the mesencephalon, leading to a predominance of a biologically-negative motivation state [29] and depression of conditioned food reflexes [37]. Under such conditions there is also a certain neuro-humoral reorganization, causing a change in metabolic processes in accordance with the new conditions in the gaseous environment.

The studies of P. Groz et al., (1972) conducted on guinea pigs kept for a prolonged period under moderate hypoxic conditions (2500 m), established, in particular, that there is a correlation between changes in the catecholamine content of the blood and urine and the acid-base balance.

Important theoretical and practical significance for aviation and cosmonautics is acquired by the development of means to increase resistance of the body to such extreme factors as acute hypoxia, acceleration, severe fluctuations in temperature, excessive physical strain, etc. As hypoxia plays a leading role in the genesis of disturbances arising with the effect of such unfavorable factors, it is perfectly logical first of all to use adaptation to hypoxia as a means of increasing resistance to oxygen insufficiency and general nonspecific resistance of the body. Methods of increasing the resistance of the body to oxygen insufficiency are acclimatization to high mountain conditions, training in pressure chambers, breathing gas mixtures "stripped" of oxygen, the use of drugs and hormonal preparations, general physical training, etc.

Comparative studies have shown that acclimatization to a high mountain climate is an extremely effective method of raising the resistance of the body to various extreme space flight factors. After 45 days in the mountains, the altitude ceiling of subjects increased 30%, and the time limit at altitude of 7500 m was doubled [1, 5, 7, 8]. Maximum working speed (on a treadmill) after mountain acclimatization increased 0.4-0.5 m/s, and endurance increased 60%. A reduction in energy metabolism was also observed with the complex effect of heat and physical loads.

Further study of physiological mechanisms of hypoxic disturbances will make it possible not only to work out specific prophylactic measures but also, according to the degree of disturbance of various functional systems of the body, to predict the adequacy of responses of the crew and the nature of their performance of flight duties.

The effect on the body of high concentrations of oxygen. Interest in studying the effect on the body of high concentrations of oxygen has increased in connection with the use of pure oxygen in the clinic to treat patients, in aeronautics and cosmonautics for pilots to breathe, as well as in underwater submersion for divers to breathe. Bert [94] was the first to establish that oxygen in high concentrations can have a damaging effect and to study the toxic effect of oxygen on the body. He found that the primary toxic effect of oxygen in high concentrations is confined mainly to irritation of respiratory passages and symptoms in the form of stagnation of the blood, edema of the lungs, bronchopneumonia with secondary disturbances of cardiac activity and circulation. Later, in a great deal of material, the etiological role of oxygen in the development of these disorders was shown experimentally.

Beginning in the 1930's in the Soviet Union, under the direction of L.A. Orbel', a large group of his scientists performed a series of basic experiments in physiological justification of the use of oxygen in dives to great depths (B. D. Kravchenskiy, S. P. Shistovskiy, 1936; S. I. Prikladovitskiy, 1936, 1940; A. G. Zhironkin, 1940, 1962, 1971; A. F. Panin, 1958, 1964; P. A.

Sorokin, 1963, and others). Other authors have also studied the toxic effect of oxygen (N. A. Savitskiy, 1940; Z. S. Gershenovich et al., 1949, 1960; N. N. Sirotinin, 1952; G. L. Zal'tsman, 1964; N. A. Agadzhanyan et al., 1967; A. M. Genin et al., 1967). Many years of observation by Soviet and foreign authors in the clinical field and in aeronautics indicate no significant disturbances with the brief use of pure oxygen under normal or reduced atmospheric pressure. This indicates, on one hand, the important significance of the time factor in the pathogenesis of oxygen poisoning, and on the other, the complete functional reversibility of changes developing during the first hours of breathing pure oxygen.

In connection with the wide use of oxygen in medical practice and aeronautics, in recent years studies have been conducted to reveal the pathogenetic effect on the body of pure oxygen and develop prophylactic measures directed / 25
toward ensuring safe breathing of high concentrations of oxygen. It has been shown that increased relative humidity at high temperatures significantly increases the toxic effect of oxygen, as does the simultaneous effect of high concentrations of oxygen and acceleration. After flights at high altitudes, pilots breathing pure oxygen revealed post-flight changes in the lungs of the "basal collapse" type.

In the studies of Comroe et al., [98] minimal time for the development of side effects after breathing 100% oxygen was 7 hours. These changes were detected even with brief flights and were connected with infections, the position of the pilot in the seat, wearing a pressure suit, etc.

It is interesting that breathing pure oxygen while performing muscular work accelerates the development of oxygen intoxication.

Despite the large number of works dealing with the effect on the body of high concentrations of oxygen, many questions remain unanswered. Especially inadequate are data on threshold values of partial pressure of pure oxygen causing a toxic effect. There is still no single opinion on maximum permissible

concentrations of oxygen in the air of manned sealed cabins with various barometric pressures; "safe" lengths of time (with regard to oxygen poisoning) which can be spent in such an environment have not been established, etc.

At the same time, practical inquiries of cosmonautics, in particular, the use of pure oxygen for breathing in life support systems, have necessitated numerous studies in this direction in recent years.

Among US physiologists the opinion is widespread that in craft launched into flight for not more than 2 weeks it is advisable to create and maintain a gaseous environment composed of pure oxygen [121]. These recommendations were carried out in the "Mercury," "Gemini" and "Apollo" spacecraft projects. In the "Apollo" craft, the life support system makes it possible for astronauts to wear both ordinary clothing and pressure suits during the flight. This system uses pure oxygen at a pressure of 258 mm. Hg and was intended to serve a 7.65 m^3 manned pressurized cabin (the volume of the "Gemini" cabin was 2.265 m^3, that of "Mercury" 1.42 m^3). As indicated by Nonoshita (1964), it was first suggested that a two-gas mixture (oxygen and nitrogen) be used, but weight limitations, the desire to simplify the gaseous environment control system, reduced air leakage from the cabin and medico-biological data showed that pure oxygen with a reduction in pressure is most rational.

However, recently the Manned Spacecraft Center in St. Louis (USA) has again turned to considering the selection of parameters of a gaseous environment for prospective spacecraft.

Widely discussed is the use of a gas diluent in manned cabins. In the opinion of American specialists, a prolonged stay in an atmosphere of pure oxygen with pressure of 258 mm. Hg causes a change in the mitochondria of the liver and kidneys without clinical symptoms of any functional disturbance of such organs.

Despite existing literature information, questions of physiological justification of an oxygen regime in manned spacecraft cabins are also still in- /26

sufficiently studied to make final conclusions or give recommendations, especially with regard to prolonged space flights.

As literature data indicate, increasing oxygen tension in the blood has a complex effect on the body. The nature and degree of changes developing in the activities of various systems and organs depend on partial pressure of oxygen in inspired air and the length of the effect. In prolonged (many days) experiments on animals it has been shown that high concentrations of oxygen with total pressure of 760 mm. Hg reduce oxygen consumption, decrease the capacity of brain tissue for oxidation and related phosphorylation. Along with disturbance of metabolic processes, changes are noted in immunological properties of the body; in particular, there is a sharp drop in the biosynthesis of antibodies and nonspecific resistance to endotoxin.

Remaining in an atmosphere containing 53% oxygen (pO_2 400 mm. Hg) for many days causes in man a slight increase in minute volume of respiration (MVR), consumption of oxygen, release of carbondioxide, a reduction in expiratory reserve volume and a decrease in the diffusion capacity of the lungs. In an atmosphere containing 40% oxygen (pO_2 300 mm. Hg), no important changes in gas exchange or external respiration are observed [1, 3, 4].

Noteworthy are the many years of observation by L. A. Palosh et al., who not only were the first to pay attention to the functional unity of the respiratory system, blood circulation and coagulation of the blood, but also experimentally showed the important biological role of oxygen in regulating hemocoagulation. In particular, it was shown that spending a prolonged period in an atmosphere with an increased oxygen content causes important changes in the picture of serum proteins and disturbs coagulation and anticoagulation systems of the blood. The authors connect these changes with the direct effect of high concentrations of oxygen on various functional systems.

In brief experiments it has been established that under the effect of oxygen thrombin is deactivated, but prothrombin shows significant resistance

to it. Disulfide groups of prothrombin are regnerated by cysteine, but prothrombin loses activity. In turn, heparin is able to prevent the oxidation of thrombin [118]. To study more deeply the state of coagulation and anticoagulation systems of the blood, long experiments have been conducted with human participation. It has been found that during a prolonged period in a hyperoxic gaseous environment, coagulability of the blood changes toward hypocoagulation [3]. The hypocoagulation effect of prolonged hyperoxia was indicated by a relative increase in the time of recalcification of plasma, a reduction in the concentration of fibrinogen, an increase in the time of blood flow and coagulation. The nature and degree of such changes depend on the length of the effect and the partial pressure of oxygen.

At the present time ever-increasing amounts of data are being accumulated that even a very moderate rise in partial pressure of oxygen in the gaseous environment during a prolonged effect causes certain unfavorable shifts in the body. In an experiment using a large number of different animals (rats, / 27
mice, guinea pigs), after a 24-day period in a gaseous environment with an oxygen content of 33-35%, a number of histochemical and biochemical shifts were noted in the body, in particular, a significant increase in the amount of ammonia in brain tissue and reduced resistance of the body to toxic concentrations of oxygen.

All this necessitates an extremely careful approach to determining the upper limit of partial pressure of oxygen in the atmosphere of spacecraft cabins and gives basis for assuming that it must not significantly exceed the normal pO_2 level in the atmosphere.

The effect on the body of hypercapnia. Thanks to fundamental studies conducted in the physiology of respiration, at the present time the biological significance of fine control of the carbon dioxide level in the blood which exists in the body is sufficiently well studied. Important regularities, established in relation to the role of carbon dioxide in oxygen transport and the restoration of vital functions, have indicated that carbon dioxide is one

of the important humoral factors in the regulation of physiological processes.

In the literature there is sufficiently complete illumination of the physiological significance of carbon dioxide. A detailed summary of the works of Soviet and foreign authors investigating the effect on the body of high concentrations of carbon dioxide is given in monographs [61, 85].

Analysis of experimental data indicates that organisms living on our planet have great adaptive resources to the effect of high concentrations of carbon dioxide. This is probably due to the fact that, throughout several geological epochs, life on Earth developed in an atmosphere containing a high amount of carbon dioxide.

In connection with man's mastery of outer space and study of the ocean depths, the question of permissible concentrations of carbon dioxide in an artificial atmosphere of sealed cabins arises anew. In the opinion of the majority of researchers, the atmosphere of sealed compartments must be completely free of carbon dioxide. However, some experts recommend using the excess to regenerate oxygen in photosynthesis systems, to prevent hypocapnia and even to weaken the damaging effect of cosmic radiation. The possibility of using a gaseous environment in manned aircraft cabins as a corrective means to prevent functional disorders arising during weightlessness and hypokinesia has also been discussed.

It must be noted that information existing in the literature has been obtained, in the overwhelming majority of cases, in brief experiments. At the same time, researchers in recent years have shown that in a prolonged period in an altered gaseous environment, the time factor is of independent biological importance. As the result of experimental research conducted by Ye. A. Kovalenko et al., on animals, using the polarographic method, it was established that the addition to ordinary air of 5% (and especially 10%) carbon dioxide significantly increases pO_2 in brain tissues which can sometimes be the same as with breathing pure oxygen. In rats breathing air, pO_2 of the

brain was 32±1.4 mm. Hg and with the addition of carbon dioxide it increased to 72±1.4 mm. Hg, while even in breathing pure oxygen, pO_2 rose only to 70±2.7 /28
mm. Hg. An analogous picture has also been observed in dogs. It was interesting to trace how the addition of carbon dioxide affected the oxygen regime of the brain while reduced concentrations of oxygen were being breathed. It was shown that in all such cases, carbon dioxide increases oxygen tension, sometimes so intensively that the pO_2 level in the brain is normalized.

The logic of this study demanded explanation of one more important question: how is the oxygen supply of the brain affected by a sharp reduction in the level of carbon dioxide in the blood during hyperventilation? This is important, as almost all stress situations are to one degree or another accompanied by hyperventilation. It has been established that severe hyperventilation of air leads to a reduction in the normal pO_2 level of the brain and heart almost 50%. What is more, even hyperventilation of pure oxygen caused a reduction of pO_2 in brain tissues from 32±1.2 to 20 mm. Hg [75]. However, all these data were obtained in brief tests. The combined effect on the body during prolonged insufficiency of oxygen and excess of carbon dioxide is extremely complicated.

The pronounced compensatory reactions of the body observed during the initial period spent in a hypoxic-hypercapnic environment are achieved at too high a price and are connected primarily with great expenditures of energy in hyperventilation. As shown by the results of experimental studies, the addition of carbon dioxide to a hypoxic mixture increases the supply of oxygen to tissues a little, but in extreme hypoxia (less than 8% oxygen) it is not able to increase, but just the opposite, can depress ventilatory reactions to carbon dioxide. With high concentrations of carbon dioxide (over 6%), respiratory reactions to hypoxia in man are weakened or disappear.

V. V. Strel'tsov [83] repeatedly noted that at high altitudes, during the effect on the body of acute insufficiency of oxygen, the addition of carbon dioxide worsens the condition of the body, as under such conditions the value

of oxygen is much greater than carbon dioxide.

As is known, the functional state of the body in an altered gaseous environment is largely determined by the interaction between biologically active respiratory gases — oxygen and carbon dioxide. P. M. Al'bitskiy, detecting the "reverse" effect of carbon dioxide, suggested that underlying observed changes is antagonism between carbon dioxide and oxygen, i.e. a fight for possession of hemoglobin [12]. Brief experiments established the presence of a certain zone where oxygen and carbon dioxide are not in antagonistic, but in harmonious ratios, ensuring in a certain time under such conditions an optimum course of oxidizing processes in tissues. However, the very factor of prolonged existence under conditions of an altered gaseous environment acquires independent biological significance in the development of physiological shifts directed toward better adaptation of the body to the environment. From that it follows that the physiological relation between oxygen and carbon dioxide is perfectly validly taken into consideration by the authors in all the above noted works dealing with hypoxia and hyperoxia.

Analysis of literature data indicates the existence of an inverse relation between permissible concentrations of carbon dioxide in closed pressurized compartments and the length of its effect. /29 For purposes of determining maximum permissible concentrations of carbon dioxide in the atmosphere of sealed cabins, N. A. Agadzhanyan et al., conducted long (10 days) experiments with human participation under conditions of normal and reduced (405 mm. Hg) barometric pressure.

Studies showed that with pCO_2 of 23 mm. Hg (3%), complete renal compensation of respiratory acidosis was observed and rather rapid reversibility of functional shifts during the aftereffect period. A hypercapnic gaseous environment with pCO_2 of 30 mm. Hg or more in the first few days alone caused significant changes in functional systems of the body: it increased minute volume of respiration and basic metabolism. Partial pressure of carbon dioxide in alveolar air increased 8-11 mm. Hg and oxygen 25-32 mm. Hg. On the

3-5th day in an atmosphere with a high carbon dioxide content, a gradual weakening of ventilator reaction was noted, a slight reduction of basal metabolism and related oxygen consumption. Changes in external respiration observed under such conditions correlate with data on blood gases and acid-base balance. In a hypercapnic environment, the active reaction of the blood shifted toward acidosis. Metabolic disturbances developed sooner, the higher pCO_2 in the chamber. Symptoms of decompensation began to appear only with pCO_2 greater than 38 mm. Hg [86].

Remaining in an environment with pCO_2 greater than 30 mm. Hg also causes an increase in coagulation activity of the blood in man, a depression of the phagocytosis process, repression of the full biological value of lymphocytes and a reduction in the concentration of sialic acids in blood serum responsible for immunoreactivity of the body.

Comparative analysis of experimental studies showed that, with the same concentration of carbon dioxide in the atmosphere of a manned cabin both in ground and in altitude experiments, the direction of changes of physiological indices is the same.

A long time is needed to normalize functional shifts, proportional to the time spent under hypercapnic conditions. Several biochemical and immunological changes in the blood are, therefore, maintained for several weeks after hypercapnia. During this period the general reactivity of the body is reduced in relation to various kinds of extreme effects, as during a prolonged period in a hypercapnic environment, carbon dioxide loses its regulatory significance, changing from a "most important component" of the internal environment of the body into a harmful and dangerous agent [12]. Functional shifts developing here reduce working capacity and disturb the adequacy of bodily reactions to prolonged extreme effects.

As the result of studies conducted to determine the general reactivity of the body, it has been established that the time limit for man at an altitude

of 7500 m after a prolonged stay in a pressurized chamber at pCO_2=23 mm. Hg was increased, and with a further increase in the carbon dioxide content, just the opposite, a progressive reduction of altitude resistance was observed. Data on tolerance of acute hypoxia agree with experiments conducted in a centrifuge and with studies of tolerance of heavy physical loads [2, 3, 10]. /30

Experimental studies have shown that with evaluation and standardization of parameters of the gaseous environment in manned pressurized cabins, the maximum permissible concentration of oxygen, carbon dioxide and other gases can be determined not only by changes in physiological reactions of the body during a prolonged period in an artificial atmosphere, but also by results characterizing tolerance of extreme effects after different lengths of time spent in a particular gaseous environment.

Limits of permissible oxygen and carbon dioxide concentrations of respiratory gases in manned pressurized cabins must be determined primarily by the length of time to be spent there. Available data, on one hand, reveal the wealth and breadth of the reserve adaptational resources of the body in the fight against extreme effects, and on the other hand, it is clearly demonstrated how complicated and at what cost functions are regulated when the uniformity of the environment is disturbed.

Total pressure of the gaseous environment and its changes have long attracted the general attention of physiologists since man's first attempts to master mountain and air altitudes and the depths of the sea. As a hypobaric atmosphere can be used in spacecraft cabins, the question again arises of whether the body is affected by an unusual level of total barometric pressure. In the middle and the end of the last century, when barotherapy was practiced purely empirically on a large scale, false opinions were formed on that count. According to such opinions, raising and lowering environmental pressure acting on the surface of the body causes a corresponding movement of the blood (to the periphery of the body with a vacuum and to its depths with increased pressure), i.e. altered pressure entails shifts and disturbance of

general hemodynamics. Such concepts were subjected to exhaustive analysis and criticism in the works of V. V. Pashutin [67]. He showed their complete groundlessness and established without doubt the fact that an unusual level of total pressure in the environment, provided it equally affects the entire surface of the body and the lungs, causes no mechanical movement of the blood in the vascular system or of lymph in the lymphatic system. However, on this basis we must not conclude that the total level of barometric pressure (with normal partial pressure of oxygen) has no physiological significance for the body.

First of all, it cannot categorically be asserted that total pressure on the body and all its tissues is, of itself, absolutely indifferent for physiological processes. The arsenal of physiology includes data indicating so-called mechanonarcosis of lower animals with extremely high total pressure [36]. If such a phenomenon actually exists, then logic cannot deny the physiological significance for the body of the total pressure under which it exists.

Secondly, related to total pressure, in particular, to its reduced level, is the physiological significance of an inert gas — a diluent of oxygen — particularly the physiological significance of nitrogen. In the absence of a diluent gas, breathing pure oxygen creates difficulties in maintaining the volume of alveolae and prerequisites for the development of atelectasis. /31

Thirdly, an altered level of total pressure in the gaseous environment also means its altered density. The practice of deep water diving and related experimental studies shows that with a high total pressure hypoxic disturbances can develop because of the increased density of the gaseous environment and the breathing difficulties it causes. Comparative experiments breathing air and helium-oxygen gas mixtures during intensive muscular work also indicate that the density of the gaseous environment, breathed by man, can be a factor limiting physical working capacity [32].

The transition of man to a rarefied atmosphere with reduced density also requires certain physiological reorganization of the work of the respiratory apparatus [70].

Fourthly, an altered total pressure level in the gaseous environment of man affects water losses from the surface of the body and upper respiratory passages. An increase of water losses in a rarefied atmosphere is of no little importance physiologically.

Finally, the fifth reason, due to the possible physiological effect on man of unusual total pressure of the gaseous environment, concerns its altered heat properties and the effect on the heat balance of the body. It must not be forgotten that these properties depend on the composition of the gaseous environment and corresponding characteristics of its component gases, but if an environment with a certain composition is meant, then its heat conductivity and heat capacity are directly connected with total pressure and density. In an environment with reduced pressure, when the air is rarefied, in accordance with its reduced density, heat conductivity and heat capacity are also reduced, which with low, ordinary and moderately increased temperatures reduces the release of heat by means of conduction. At the same time, the release of heat by evaporation in a rarefied atmosphere must increase because it, as a rule, contains a lesser amount of moisture [19].

The development of cosmonautics again brought the attention of physiologists to the problem of forming an atmosphere in closed cabins.

Factors of the total pressure of the gaseous environment and its changes must be considered in three aspects. The first is the search for and justification of such parameters of an artificial manned spacecraft cabin atmosphere which would preserve the health and a high level of working capacity of the cosmonauts in flight. The second aspect is study of the effect on the body of these factors with regard to possible accident situations during flight. And, finally, the third aspect of the effect on the body of total pressure of the

gaseous environment and its changes is connected with the necessity of resorting to significant deviations of these basic gaseous environmental parameters from ordinary and optimum values in order to ensure the well being of the cosmonaut and his activity in exiting the spacecraft cabin into open space.

To prevent decompensation disorders, P. M. Gramenitskiy et al. conducted studies to justify a rational gaseous environment for spacecraft cabins, ensuring the safe transition to pressure in a space suit. It has been shown that a gaseous environment with total pressure of 405 mm. Hg and normal partial pressure of oxygen, after spending a day in it, provides man safe transition to the lowest practically permissible pressure in a space suit, 170 mm. Hg. /32

Besides justification of this gaseous environment, studies have led to the general conclusion of the advisability of using a two-component atmosphere with reduced total pressure. Of course, with the use of any specific variants of such a hypobaric atmosphere, partial pressure of oxygen is intended to be maintained at normal or near-normal levels. Periodic moderate reductions of partial pressure of oxygen and increase of partial pressure of carbon dioxide in the atmosphere of the spacecraft cabin could be useful during a space flight for maintaining working capacity and general bodily resistance of the cosmonaut. If this problem, now in the state of systematic experimental development (N. A. Agadzhanyan, et al.), is receiving convincing positive solution, the use of such a dynamic atmosphere with preprogrammed periodic fluctuations of pO_2 and pCO_2 will undoubtedly find a place in space flight practice. It must, however, be assumed that in such case fluctuations of pO_2 and pCO_2 will be most favorably ensured by maintaining total pressure of the gaseous environment at a relatively unchanged level.

On the basis of the above, what conclusion can be made regarding the effect on the body of an altered total pressure level in the gaseous environment? The use of any significantly increased pressure in spacecraft cabins is excluded, as this is disadvantageous from a physiological and all the more from a technical point of view. Therefore, it can be either a normal or re-

duced total pressure in the cabin. The use of a two-gas environment in spacecraft cabins with normalized pO_2 and total pressure reduced to approximately half the ordinary level, as facts show, conceals no threat to the condition of the cosmonaut or his working capacity. This follows from the above noted analysis of possible physiological effects on the body of reduced (within stated limits) total pressure and is also confirmed by two unquestionable factual proofs. The first of these is the continued life of a large number of people in high mountain regions at altitudes of 3-4 km. The atmosphere of a high mountain area differs from variants of a rarefied atmosphere considered for spacecraft cabins in that under mountain conditions the hypoxic factor has a continued effect, with which high mountain acclimatization is also primarily connected.

The second stricter proof of the absence of a negative effect on man of reduced total pressure of the gaseous environment is presented by special experimental studies performed on people spending a long period of time in a pressure chamber in a rarefied atmosphere with normalized partial pressure of oxygen. Such studies included an experiment in which subjects spent 62 days in a pressure chamber with a vacuum corresponding to an altitude of 7000 m (total pressure 308 mm. Hg with pO_2 of 160 mm. Hg), evidencing no significant functional shifts [3] dangerous for human health.

Still more convincing are experiments performed in past years and repeated and extended at present with subjects spending a month in a pressure chamber with total pressure of 405 mm. Hg in an atmosphere with normal and moderately reduced pO_2 (105-125 mm. Hg), containing no carbon dioxide or else enriched with such to 22 mm. Hg. These experiments, which showed a number of different adaptational physiological shifts in the body of the subjects, imply that total pressure of the gaseous environment reduced to 405 mm. Hg of itself does not have an appreciable negative effect on the human body. Reduction of the total pressure in the cabin will lessen the danger of explosive decompensation in case of a seal failure of the cabin, reduce gas leakage from the cabin, decrease the weight of the cabin and the expenditure of energy in its

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ventilation, improve thermoregulation and create more favorable conditions for entering and exiting the cabin in space.

These materials indicate that the creation of an artificial sealed-cabin atmosphere is a complex set of physiological and technical problems requiring comprehensive study for their solution.

Prospects for the development of cosmonautics require further research to study the effect on the human body of different variants of a gaseous environment, differing from the normal atmosphere in total pressure and partial pressure of respiratory gases (oxygen, carbon dioxide) in order to establish the relation between the gaseous environment and general reactivity of the body.

N75 14407

The Internal Nature of Functional Disturbances Arising in the Body under the Effect of Extreme Factors

The main purpose of space physiology is to study the effect on the body of both individual space flight factors and their combination in order to work out measures directed toward preventing or reducing the effect of harmful factors and increasing the resistance of the body. This can be achieved only by sufficiently deep physiological analysis of the effect on the body of a complex of space flight factors and revelation of general regularities in the interaction between the body and unusual (extreme) factors of the environment. The present work is connected with this latter important problem, which is of serious theoretical interest and is the basis for solving the mentioned practical problems. Its authors, concerned with studying the effect on the body of different factors of the gaseous environment with various effects and various experimental conditions, encountered the same fact: with an identical degree of compensation by the body of a particular external factor, as a rule, functional shifts and functional disturbances in the body differ in expression and force.

It is known that with exactly the same force of an external factor, functional shifts in the body of individual persons and animals of the same species can be different. This is often due to differing individual resistance of the body to corresponding influences. These differences, evidently, have various foundations. Here a role can be played by individual constitutional-morphological characteristics of the body (for example, an abundance of fat in the body which predisposes development of decompression disturbances during pressure drops) or latent defects of the cardio-vascular system or lungs which can be a condition determining reduced resistance of the body to hypoxia. Characteristics of individual resistance to unusual effects having such basis are important in a practical sense, but are not of significant theoretical interest and can shed no light on general regularities in the interaction between the body and unusual factors. /34

Much more important are differences in individual resistance having a purely physiological foundation and connected with protective reactions of the body, directed toward compensation of the external factor. (We are not going to concern ourselves with general, so-called nonspecific resistance of the body, as consideration of this still largely unclear matter would lead us away from the main theme of the work). Characteristics of individual resistance are usually due to different value and expression of these protective compensatory reactions in individual persons or experimental animals and, therefore, the degree of compensation by the body of the external factor is also different. For example, with the same reduction of partial pressure of oxygen in the environment, because of peculiarities in the reactions of the respiratory apparatus and differing volumes of pulmonary ventilation, partial pressure of oxygen in alveolar air and arterial blood can be reduced to different levels. The hypoxic factor at this first stage of its effect, therefore, can be more or less completely compensated by the body, and related functional changes in the body can vary. Equally, under the effect of protective thermoregulatory reactions, various degrees of general internal hyperthermia of the body can also develop and in accordance with such, there will be various functional shifts and functional disturbances.

Such facts are generally perfectly clear, although a deeper approach to them shows they are far from being so simple and require careful analysis. However, the logic of this explanation of different individual resistance of the body to unusual facts here is quite simple: the more completely the body compensates the effect of the external factor, i.e., the greater the extent to which it averts shifts caused by this factor in its internal environment, the less pronounced will be functional changes and functional disturbances under this effect.

I. I. Dvorzhak, M. M. Moravek and Ya. I. Tsmiral, in studies on humans, established that with the development of hypoxemic conditions caused by breathing gas mixtures combined with oxygen, by "ascents" in a pressure chamber and by the rerespiration method, in cases when a completely identical level of oxygen saturation of the blood is noted in individual subjects (i.e. there is the same degree of compensation of the external factor), functional disturbances are of differing severity. K. Kh. Kunchev revealed an analogous fact in a great amount of material in conducting standard tests with subjects (pilots and students) spending 30 minutes in a pressure chamber at an "altitude" of 5000 m. Different tolerance of such conditions by individual persons was ascertained with the very same expression of respiratory and cardio-vascular /35
compensatory reactions and with the same degrees of oxidation of the blood.

It has been shown that with the very same (reduced) level of oxygen pressure in brain tissues of animals during altitude hypoxia, the depth of functional disturbances of the body is also different; this occurred not only with moderate and medium force hypoxic effects — at "altitudes" of 5-8 km — but also with sharply pronounced hypoxia — at "altitudes" of 9-10 km [47].

P. M. Gramenitskiy et al. detected functional disturbances, differing in severity, with the same degree of elevated partial pressure of oxygen in brain tissues of animals during the general toxic effect of oxygen under increased pressure, as well as functional shifts, varying in expression, in the body of men and animals during the effect of elevated surrounding temperature,

when the general level of internal body temperature; i.e., the degree of general hyperthermia, was the same [31].

Therefore, there is one fact common to all these studies: different degrees of functional changes and disturbances in individuals, despite identical indices of compensation by the body of the external factor.

It must be noted that if we consider such general indices of compensation as oxygen saturation of arterial blood under hypoxic effects or the internal temperature of the body during the effect of elevated surrounding temperature, then this fact cannot be surprising. Regarding hypoxic effects, it can be assumed that with an equal degree of oxygen depletion of arterial blood the body can, figuratively speaking, distribute the oxygen-depleted blood variously among organs and this can be the cause of variously expressed functional disturbances. Analogously, with heat effects it can be assumed that, with the same internal temperature (temperature of arterial blood), various temperature shifts occur in different organs and this can be related to differences in the degree of functional disturbances. Such situations can, undoubtedly, be encountered in reality and as applied to them these explanations are very probable.

However, it is not only a question of such cases when only the above named general indices of compensation by the body of unusual external factors are identical. The above data indicate that if we turn to deeper indices, then it is found that the essence of the matter remains the same. Indeed, according to material obtained in the laboratory of Ye. A. Kovalenko, in case of hypoxic effects, even with completely identical absolute values of oxygen tension in tissues of the brain, different functional disturbances are observed in the central nervous system and the body as a whole. This also occurs in hyperoxia. In experiments with heat effects, P. M. Gramenitskiy et al. studied not only general temperature shifts in the body, but also thermotopography of organs (in man — thermotopography of the skin); it was also noted that despite identical general and local temperature shifts in the body, functional disturbances in individual persons during hyperthermia can be different. There- /36

fore, the fact is that even when the degree of compensation of an external factor is the same at all levels, even down to the organ-tissue level, the degree of functional disturbances is different.

Analysis of this fact was conducted by authors in various directions. I. I. Dvorzhak, M. M. Moravek and Ya. N. Tsmiral developed a complex model on analog computers reproducing changes in pulmonary ventilation and shifts in arterial oxygen saturation during hypoxic conditions. Data obtained from this model agreed with results of actual human experiments. The authors concluded that disturbances in human working capacity during hypoxia are connected both with the direct effect of the hypoxic factor and with the reactions of the central nervous system under such conditions.

K. Kh. Kunchev gave special attention to cardio-vascular reflexes as a criterion of adaptive resources of the body to extreme effects, including hypoxia. It was found that this criterion proved itself significantly: with positive indices of corresponding functional tests, a relatively high resistance of a given individual to these extreme effects is noted. At the same time in a number of cases with identical values of this criterion functional shifts, caused by extreme factors, particularly hypoxia, can be different.

In the laboratory of Ye. A. Kovalenko were studied tissue respiration processes during hypoxic effects [58]. Theoretically important changes were introduced into the known Warburg method: in the manometric vessels in which the tissues were placed, the usual level of oxygen tension for such studies (160 mm. Hg) was not maintained; instead, the reduced pO_2 which was recorded in corresponding tissues of the body during hypoxic states was used. It was shown that as the result of the effect of hypoxia on the body (in combination with hypercapnia), tissues of the body acquire the ability to absorb and utilize quite effectively oxygen from the environment, even at low pO_2, while tissues of control animals under such conditions show no such ability. At the same time both in tests and in the control individual, differences were noted in animals with regard to the intensity of tissue respiration at the very same

pO_2 values in the environment. These data can serve as a direct explanation of the basic fact we have been discussing — functional disturbances which differ in severity with an equal degree of tissue hypoxia.

The significance of reactions at the cellular-tissue level during the effect of unusual factors is also indicated by experiments with heat effects. Data have been obtained on the resistance of animals (dogs), adapted and not adapted to heat, to critical heat effects, leading to death. The dogs were placed in a heated chamber (temperature 50°, relative humidity 60-70%) and kept there until death. It was shown that the survival of animals, previously adapted to heat, under the above conditions, exceeds that of control dogs, not adapted to heat, 1½-2 times. It was especially significant that control animals died at a significantly lower degree of internal hyperthermia. Therefore, as the result of adaptation to heat, organs and tissues of animals acquired the /37 ability to tolerate high degrees of elevated temperature. From that it follows that resistance of the body to heat depends not only on the degree of compensation of the temperature factor, but also on the reaction of cells and tissues to hyperthermic conditions, which are created in the internal environment of the body. This can also, to a certain degree, explain the differences in the severity of functional disorders with the same indices of compensation by the body of the external factor.

It would, however, be a mistake to explain this fact only as different resistance of cells and tissues to internal changes in the body, occurring under the effect of unusual external effects.

The basis for different degrees of functional changes in individual persons with the identical force of the external factor is already created at the first stages of interaction with this factor, when significant shifts in the internal environment of the body have not yet developed at the tissue level. In order to explain this, it is necessary to turn to general regularities in the interaction between the body and unusual factors, particularly, the factors of an altered gaseous environment.

Fundamental basic materials in this area were obtained by P. Behr and leading figures of Russian physiology and pathology, I.M. Sechenov, V. V. Pashutin and their successors. An especially thorough and comprehensive analysis of the interaction between the body and various unusual factors of the gaseous environment was undertaken by M. P. Brestkin et al. [20]. As a result, general regularities in the interaction between the body and these factors were revealed. We shall not reproduce in detail these regularities, but only touch on basic propositions directly related to the question under discussion.

Under the effect of unusual factors of the gaseous environment, in particular, altered partial pressures of oxygen, protective compensatory reactions of the body are developed by mobilization of its physiological reserves. This mobilization of reserves is based on the principle that, besides intensification of the activity of organs and systems carrying out compensation of the unusual factor, there is an inhibition of functions having no vital significance in this case. Thus, for example, during hypoxia, along with activation of respiration and blood circulation, there is a pronounced inhibition of the digestive function, which can be verified by testing the activity of salivary and stomach glands and motor-evacuation work of the stomach and intestine. These functions during hypoxia are always depressed to a greater or lesser degree. However, it would be a mistake to treat these functional shifts as purely pathological disturbances of corresponding functions.

In analyzing the essence of these functional changes, it is shown that they are an integral component of the general adaptive reaction of the body to hypoxia and help counterbalance this factor, releasing additional reserve resources, in particular, in the circulation system and in supplying oxygen to such vital formations as the central nervous system, cardiac muscle and respiratory apparatus. Thus, compensatory reactions during hypoxia are based on the principle of dominant activity of the cardio-vascular and respiratory systems.

In the nervous system a functional constellation of centers carrying out protective reactions is formed with a simultaneous drop in functional activity of other nerve centers related to functions having no decisive significance in this situation. This principle extends to various kinds of working activity in man and behavioral acts in animals. In testing the functional capacity and working ability of the body under hypoxic conditions, some deviations or other from normal are also detected. Here also the degree of these deviations depends on two basic causes: first, on how essential and vital, under given conditions, is the particular working (in animals — behavioral) activity, how reinforced it is by corresponding training, and, secondly, how strong are the physiological reserves of the body guaranteeing its fight against unfavorable external effects. The stronger these reserves, the less the working capacity (functional capacity) of the body suffers and the more important for the body this activity is under such conditions, the more reinforced and automatic it is, the lower the degree of changes occurring in it under hypoxic conditions. From the above it follows that depending on these two causes, functional disturbances in the human and animal body can be essentially different, even with the very same index of compensation of the hypoxic factor — an identical degree of oxygen saturation of arterial blood. It can, therefore, be said that this is the first functional level of compensatory reactions when, depending on the physiological reserves and the nature of the studied activity, and if the intimate side of the matter is taken, depending on relations between corresponding centers in the nervous system, variously expressed general functional changes and disturbances arise in the body.

If now we turn to the cellular-tissue level of compensation of hypoxic effects, then differences in functional shifts during hypoxia can be determined on this level by the difference in the blood supply of individual organs, and, therefore, also the difference in oxygen and carbon dioxide tension in tissues. Of interest to us, however, are not these cases, but those when indices of compensation of the hypoxic factor are identical at the cellular-tissue level, but functional changes in the body differ. Evidently, here the cause of such differences can already be the physiological properties of tissues and cells them-

selves — their greater or lesser resistance to the given altered conditions. As concerns hypoxia, it is well known that this heightened resistance of cells and tissues to reduced oxygen tension is one of the most important and basic components of acclimatization to oxygen insufficiency. We must, however, assume that in those people and animals which have never before encountered an external hypoxic factor, this tissue resistance to it is not the same. Probably, the main reason for this must be greater or lesser training of the body to performance of muscular work, particularly, maximum physical loads.

In addition to the above, we must note one more theoretically important situation, detected by B. A. Vinokurov in intimate analysis of the effect on the body of factors of an altered gaseous environment. Using as an example the stimulation of centrifugal nerves of the heart in dogs during identical degrees of hypoxia, hypercapnia and a combination of these effects, V. A. Vinokurov showed that the very same nerve stimuli can lead to changes in cardiac activity which are different, often unusual and not in the nature of the stimulated nerve.

The fact which emerged in our studies — varying functional disturbances in the body with identical indices of its compensation of an unusual external factor — can, therefore, underly at least three cases: unusual intercentral relations in the nervous system in interaction between the body and the factor, differing resistance to it of cells and tissues of the body and the reaction of effector organs to nerve stimuli regulating their work. This probably does not exhaust the essence of this phenomenon and it must be the subject of further research. This is stimulated by both the great theoretical interest in this matter and its evident practical significance, the possibility of accurately predicing changes in working capacity of man under unusual conditions.

N75 14408

Internal Thermotopography and Shifts in General Thermal Balance in Man under Special Heat Transfer Conditions

Heat exchange and thermal regulation comprise one of the most important problems in space physiology. Two extremely important aspects of this problem can be distinguished in connection with medico-biological support of space flights.

The first aspect, acquiring special practical importance, is connected with cosmonauts working in pressure suits in open space. Thermal regulation of man in isolated individual means of protection by a system of artificial heat removal is a special section of thermal regulation which is of important scientific and practical significance. This aspect of protection has already received interesting development, marked by extremely important scientific and practical achievements. However, a number of theoretical questions still remain poorly understood and a number of scientific and practical problems are unsolved.

The second aspect is studying possible changes in the heat regulating function of the human body occurring under the influence of the complex effect of space flight factors and conditions. Most important of these factors are prolonged weightlessness, prolonged limitation of motor activity (hypokinesia) and, finally, possible deviations in microclimatic parameters of the environment.

Thermal regulation as a very highly organized, evolutionarily comparatively young function of the body, controlled by higher sections of the central nervous system, naturally, cannot remain unchanged during the effect of such factors, all the more as it is directly connected, on one hand, with the state of metabolism and on the other, with the activity of the cardio-vascular

system and respiratory apparatus, i.e. with those physiological structures for which the effect of weightlessness, hypodynamia and an unfavorable microclimate are extremely important. From that arises the necessity, first of all, of explaining a number of related purely practical issues (for example, determination of optimum microclimatic conditions in a spacecraft cabin or in a pressure suit and necessary thermal properties of cosmonaut clothing), and, secondly, of studying changes in the heat regulatory function of man, occurring under the effect of space flight factors and conditions. A separate, extremely important question concerning this aspect of the problem is study and evaluation of temperature shifts in the human body under physical loads under limited space conditions against a general background of limited mobility and weightlessness. /40

Working out thermal regulation in space physiology must, therefore, combine experimental-analytical research (particularly on animals) with studies of a practical nature under conditions which as close as possible approach actual space flight.

The works of specialists cooperating within the framework of "Interkosmos" deal with both these directions. In the creation of favorable living conditions in the spacecraft cabin and in exiting, an important place is occupied by reliable, effective means of individual protection against the harmful effect on man of space factors. However, such equipment, protecting man from harmful external effects, can itself have an unfavorable effect, the nature and severity of which depend on the type of equipment, operating conditions and a number of other factors.

The barrier between the environment and man produced with the use of such equipment can sometimes disturb the transmission into the environment of body products: heat, moisture, carbon dioxide, carbon monoxide, amines, phenol, etc. Of these, especially unfavorable for man is the heat factor, resulting from a disturbance of heat release from the body. In particular, in the absence of a heat removal system in individual protective equipment or with its failure, the

thermal balance of the body can quickly be significantly disturbed with a sharp reduction of working capacity.

The time a man can spend under such conditions is limited because of the danger of heat stroke.

In connection with these problems of maintaining thermal balance in man in individual protective equipment, considered from a medico-biological point of view, most urgent are, in our opinion, the following directions of research:

1. Detailed study of the thermal condition and physiological mechanisms of maintaining thermal balance in the human body in individual protective equipment during unfavorable microclimate conditions.

2. Scientific justification and development of physiologically acceptable effective heat removal systems, ensuring preservation of the thermal balance of a man working in individual protective equipment.

3. Theoretical justification and development of effective means of raising the thermal resistance of the body.

4. Justification of hygienic standards and requirements for individual protective equipment which will ensure maintenance of thermal balance in man and his high working capacity.

Solution of these problems will make it possible to diagnose accurately the thermal condition of man and evaluate objectively his reserve resources during a thermal load, i.e. to predict the dynamics of heat stress and changes /41
in working capacity; to preserve thermal balance and maintain high working capacity during significant energy expenditures; to raise heat resistance of the body substantially; to provide scientifically justified standards and to formulate requirements for individual protective technology and methods for its physiological-hygienic evaluation.

Needed for successful conduct of studies and for obtaining comparable results is a great deal of work to improve and unify methodological procedures most often used in experiments. In connection with the extensive volume of medico-biological information obtained in the course of experimental research, it is extremely urgent to develop a method of automatic collection and analysis of data, recorded in the course of an experiment.

In discussing the problem of the thermal condition and physiological mechanisms of thermal regulation of the human body in unfavorable microclimate conditions, it is necessary first of all to point out the great difficulty of solving it because of the unusual complexity of the thermotopographic picture of the human body.

There are extremely complicated temperature ratios between individual organs in the internal environment of the body. These ratios and the temperature of individual organs as well, although differing in variability in the normal body, vary only within certain limits and, therefore, are, to some degree, constant. The term "body temperature" is very arbitrary as actually there is no one certain body temperature. What is more, each organ of the body is characterized by its own temperature regime, i.e., by temperature ratios between components of each organ.

Only arterial blood has a uniform and very constant temperature. This constancy of its temperature is a necessary condition for maintaining the normal temperature regime of various organs, which differ in their average temperature from arterial blood. The thermoregulatory system of the body, therefore, regulates not some general body temperature, but the temperature regime of organs and temperature ratios between them. Regulation of thermal exchange in organs and the temperature regime of each is inseparably connected with regulation of the thermal balance of the entire body and together with it forms a single physiological process.

For a final definition of the essence of reactions of the body to external temperature effects it is not enough to know their general mechanisms and general totals (changes in general heat production and heat transfer). It is also necessary to learn what internal changes in heat exchange in various organs are responsible for these reactions of the body.

We must also not reduce all disturbances of thermal regulation to stronger accumulation of heat in the body or greater release to the outside. In either case there can be a greater or lesser disturbance of internal temperature ratios and the temperature regime of individual organs. If disturbances develop in organs of vital importance to normal activity, they can cause extremely serious consequences [25, 26].

It must be emphasized that heat released in the body is not an energy source for biological work. It is used to maintain body temperature within the narrow temperature range characterizing homoiothermy. Therefore, the most /42
important problem in thermoregulation is removal of excess heat. Heat removal is important for improving heat exchange under various conditions.

Of course, the immediate question is scientific justification of methodological approaches to evaluating the thermal condition of man in individual protective equipment.

In order to form the correct idea of the thermal condition of the human body it is necessary to know the indices characterizing the thermal balance of the body: temperature of the body and skin, average body temperature as derived from body temperature and skin temperature, mositure loss, heat production and, finally, the heart rate as an integral index of the functional state of the body. However, none of these indices in isolation can indicate the thermal condition of the body. Even such a seemingly informative index as body temperature does not reveal the true content, retention or loss of heat in the body as the thermal balance can change during uncorresponding changes in body temperature.

Of significant importance in evaluating the thermal condition of man is average body temperature. Only this can correctly indicate the amount of heat retained (or released) by the body during a certain period of time.

Evaluating the thermal condition of man greatly facilitates the study of moisture loss. These values of effective and ineffective moisture release help to evaluate objectively the degree of heat stress in unfavorable conditions of individual protective equipment.

Later, in order to determine the state of heat exchange and thermal regulation, it is very important to know the value and dynamics of heat forming processes. This will provide data to evaluate the thermal balance of the body.

Objective evaluation of the thermal condition is, therefore, basic for the general functional characterization of man; however, comprehensive physiological evaluation is possible only by taking into consideration data on changes in a number of other leading physiological systems and subjective evaluation of man working in individual protective equipment.

In recent years, quite extensive material has been accumulated on the physiology of heat stress. In particular, classification of thermal conditions in man has been devised along with a minimal complex of informative physiological indices justified for medical control [28, 54].

These studies have made it possible to solve a number of problems concerning characteristics of heat exchange and thermal regulation of man under heat load conditions typical of individual protective equipment and to justify practical recommendations for medical control. Evidently, further studies in this direction must be devoted to expanding our ideas of the essence of the mechanisms of thermoregulation in man under various microclimatic conditions and of the origin of heat stresses.

With regard to the second direction of the problem, today physiological acceptability and high effectiveness of a contact heat release system can be considered practically resolved. A heat release system based on the conductive principle of cooling the human body is extremely effective; it can remove about 500 kcal/hr [27, 28]. /43

The third very important direction in the problem of maintaining thermal balance in man is experimental development of means to increase the heat resistance of the body. On the basis of theoretical propositions and suggested methods for diagnosing the thermal condition of man, S. M. Gorodinskiy et al. recommend a number of means and methods to increase the heat resistance of man which will expand the range of application of individual protective equipment. Thus it has been shown that:

1) by chilling a small portion of the body with a cold panel (with a coolant from -5° to -7°) measuring 0.1 m^2, continued performance of physical work (~250 kcal/hr) can be ensured for 2-2½ hours during complete thermal isolation of man. Also determined are areas of the body where chilling will give maximum effect under such conditions [56];

2) by preliminary chilling of man, reducing the heat content of the body to 28.5 kcal/kg from the initial 30 kcal/kg, the time for performing physical work under conditions of difficult heat exchange can be markedly increased [28];

3) by breathing chilled air during performance of physical work in a warm microclimate, heat resistance can be increased 1½ times, especially with simultaneous oxygen enrichment of the air [69].

It can be assumed that one of the actual paths toward raising the heat resistance of the body in unfavorable microclimatic conditions could be the use of a number of pharmacological, particularly biogenic preparations directed toward chemical and physical thermoregulation. Studies in this direction are,

evidently, very promising [52, 53].

The next problem in maintaining thermal balance in man in isolating individual protection equipment is its hygienic aspect — standardization of the microclimate of individual equipment. Evidently, such standardization is responsible for the functional state of the body.

On the basis of the classification developed [28], three thermal conditions of man are distinguished during difficult heat exchange (optimum, permissible and critical) and three microclimate zones corresponding to such conditions.

Optimum microclimate conditions are those which preserve thermal balance without pronounced stress of physiological mechanisms of thermoregulation. Such conditions presuppose the absence of an influx of heat from without and removal of heat produced by the body with optimum heat transfer structure. Air flow is taken as 0.2-0.3 m/s, as even in suits with forced ventilation it, as a rule, did not exceed these levels.

With an optimum microclimate in isolating individual protection equipment, the length of time spent in them is determined not by the thermal condition of the body, but by fatigue, the rate of development of which depends on the regime and nature of the work, the situation, etc.

When work which is limited in duration or episodical work is to be performed it is permissible to be oriented not only toward an optimum microclimate in sub-suit space, but also toward microclimate conditions when all the heat produced by the body is not removed. However, in such case it is also /44
necessary to ensure the thermostable condition of the body, even with its increased heat content.

Microclimatic conditions providing a thermostable condition of the body during heat accumulation not exceeding 1.5 kcal/kg are called "permissible." Under such conditions pronounced stress of thermoregulatory mechanisms is

noted and a change in the structure of heat transfer, appearing as increased heat loss by evaporation.

To regulate the time spent in a permissible microclimate of sub-suit space, it is necessary to be oriented toward the failure of stabilization of the thermal condition and working capacity, determined by the nature of the work performed.

When the temperature and humidity of the air in sub-suit space are beyond the limits of the permissible zone, thermoregulatory mechanisms, despite their significant stress, are not able to stabilize the thermal condition. As a result, there is a continuous increase in the amount of heat in the body and as a consequence — a critical thermal condition. Functional shifts under such conditions (called "critical") are objective indices for ending the stay in isolating individual protective equipment.

Evidently, further development in this direction must be connected with solution of such an immediate problem as the creation of a complex physiological-hygienic method of evaluating means of individual protection for man on the basis of the most informative indices. For successful solution of this problem it is necessary to use an automated system of collecting, analyzing and storing medico-biological information obtained in the course of experimental research. The creation of such a system will significantly increase the quality and reduce the time of performing such operations.

The second aspect of the problem is studying the mechanisms of thermoregulatory reactions of the body. The purpose of such studies is deeper investigation of intimate thermoregulation processes, the mechanisms of accommodative reactions to heat and especially the role of the central nervous system as a whole and several of its sections in maintaining thermal homeostasis under heat load conditions.

Some new data in this direction has been obtained which is deserving of attention. In tests on animals, regularities in the development of hyperthermia and mechanisms of thermoregulatory reactions were studied during the application of heat. It was established [109, 110] in tests on rabbits that with environmental temperature of 41-43.5°, despite pronounced thermoregulation reactions in the respiratory system and vessels in the ear, general hyperthermia develops rapidly; along with a rise in the temperature of the "core" of the body, oxygen consumption and heat production increase, thereby creating a unique closed circle of phenomena accelerating the overheating process. Experiments have shown that thermoregulation reactions in non-narcotized animals are the result of hot air on the skin and mucous membranes of respiratory passages and are increased further under the effect of the rise in temperature of the "core" of the body. Under narcosis only the role of internal temperature /45
shifts appears in the development of thermoregulatory reactions.

Special attention in a number of works has been given to the temperature regime of the brain of animals under various external conditions and those consequences resulting from temperature shifts in various areas of the brain. In chronic experiments on rabbits, with thermocouples inserted in the brain and in various areas of the body [114], the relation between the condition of vessels in the ears and the temperature of the brain was traced under various microclimatic conditions. It was established that with an environmental temperature of 10° vasodilation in the ears reduces the temperature of the brain 0.6°; with a temperature of 20° this effect is 0.3°. With high external temperature (+40°), when heat transfer is possible only by means of the evaporation of moisture, vascular reactions in the ears lose their effectiveness and the main role is acquired by thermoregulatory changes in respiration. Under such conditions its rate in rabbits increases 15 times; the temperature of the mucosa of the nose drops several degrees and remains 5° below that of the environment. At the same time the temperature of the "bulbus olfactorious" is reduced and after a short delay also that of the blood in the carotid artery, of the hypothalamus and of the entire body.

Tests have shown that, first of all, under high temperatures, vasodilation of mucous membranes of upper respiratory passages and the simultaneous increased respiration rate protect against hyperthermia and, secondly, that maintenance of the temperature of the brain at the initial level is connected with general temperature shifts in the body of the animals.

In a special work [107], effects caused by a local increase in the temperature of the preoptic section of the brain and anterior hypothalamus were studied in rabbits; a heating thermoelement was introduced into this zone by the stereotaxic method. At the same time electrodes were introduced into the sensory motor area of the cortex and the dorsal hippocampus to record biopotentials and into the medial area of the thalamus to record electric stimulation of this zone. Tests were conducted with neutral (20-22°) and elevated (27-30°) temperature of the environment. It was found that in response to local increase of temperature in the preoptic section of the brain, in rabbits the temperature in the outer surface of the ear is regularly raised and the respiration rate increased. At the same time there are typical shifts in the bioelectric activity of the tested areas of the brain: a clear tendency was found toward synchronization of the EEG. After heating of the preoptic section of the brain is terminated, the temperature of the ear drops, respiration slows down and at the same time desynchronization of the EEG is observed.

Analysis of experimental data has shown that with local elevation of the temperature of the "center of heat loss," when corresponding thermoregulatory reactions develop, the activating nonspecific system is inhibited.

A special series of studies was devoted to studying the temperature regime of the brain in various animals, its dependence on blood supply and its changes during the effect on the body of such factors of the gaseous environment as fluctuations in partial pressures of oxygen and carbon dioxide [62]. Experiments have already been made on rats, rabbits and dogs with the insertion of thermojunctions into the brain of the animals, imbedded to various depths, and with thermocouple-sounds, introduced into the abdominal section of

the aorta. During the experiments, rats were strictly confined, rabbits were placed in a special cage limiting their movement and dogs were trained to stand freely at a station. /46

Layered thermometry of the brain in all these kinds of animals under ordinary external conditions showed the existence of a constant temperature gradient from the periphery of the brain to its deeper parts. In surface layers of the brain, temperature is lower than in the deep sections. This indicates the presence of direct external temperature effects on the peripheral layers of the brain. When the temperature of the environment is reduced, when natural heat insulation of the brain is disturbed (removal of hair, skin) and when the animal is immobilized, there is a reduction in the temperature of the brain (to a greater degree in surface sections) and an increase in layered temperature differences.

The possibility of direct temperature effects on the brain is extremely important from the practical point of view and interesting on the theoretical level, as in connection with such a possibility arises the question of temperature physiological optimum for brain activity, particularly in its higher sections.

In experiments studying the effect of carbon dioxide (5 and 10% in inhaled gaseous mixtures) on the temperature regime of the brain of rabbits and dogs, a pronounced reduction of temperature was detected in the brain in all layers, evidently connected with repression of heat production of brain tissue. The reduction of temperature of the brain as a whole was greater than that of the body (rectal temperature at a depth of 7 cm), and in some cases even exceeded the reduction of temperature in the aortal arterial blood. Analogous, but significantly more pronounced effects were obtained in the simultaneous influence of rapidly increasing hypercapnia and hypoxemia. Facts obtained are noteworthy in connection with the mechanism of the effect of hypercapnia on functions of the central nervous system, as well as in connection with the general problem of the biological role of carbon dioxide. At the same time they are directly

related to regulation of the temperature regime of the brain under ordinary and altered conditions [62].

In tests on rabbits (E. Turleyska) it was proven that thermoregulatory reactions to the elevation of temperature of the hypothalamus significantly change during dehydration of the body. The development of dehydration in man disturbs thermoregulatory processes during physical loads; this is not connected with a reduction in the volume of circulating blood.

In the studies of S. Kozlovski et al., it was shown that perfection of thermoregulatory processes during physical loads to a certain degree correlates with maximum oxygen absorption by the body ("oxygen flux"). The authors assume that the difference in thermoregulatory effects is due to the peculiarities of the cardio-vascular systems and the specifics of heat transfer from the "core" to the surface.

This direction of works on thermoregulation, concerning the analysis of the mechanism of thermoregulatory reactions and temperature ratios in the internal environment of the body, is extremely useful in evaluating and interpreting results of tests on man under specific conditions simulating human life and work /46 in isolating equipment.

The most urgent issues for further research are study of the peculiarities of heat exchange and thermoregulation in man in individual protective equipment; physiological justification of microclimatic standards and values for heat loads on the human body; development and justification of means of maintaining thermal balance in man, as well as further experimental research on animals and observations of man for a deep understanding of various aspects of thermoregulatory mechanisms of the body under difficult thermal exchange conditions. Conduct of such studies will make possible still deeper understanding of thermoregulatory mechanisms of the human body during a heat load, as well as significant expansion of the limits of possible use of individual protective equipment, raise working capacity and preserve the health of workers in such equipment under extreme environmental conditions.

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Tolerance of Acute Hypoxia while Performing Operator Activity and after a Prolonged Period under Altered Gas Environment Conditions

The use of sealed cabins and modern oxygen-breathing equipment in aviation and cosmonautics makes possible long flights at great altitudes. However, under actual flight conditions the possibility is not eliminated of leakage of atmosphere from the cabin or failure of the oxygen-breathing equipment, as the result of which members of the crew could be exposed to acute hypoxia. This is why further study of physiological mechanisms of hypoxic disorders, especially in the performacne of operator activity, is extremely urgent.

For the practice of aviation and space medicine it is important not only to know the length of time sufficient working capacity and active consciousness are preserved in man during various degrees of hypoxia, but also how the resistance of the body to extreme effects is altered after a prolonged period in an artificial atmosphere with various oxygen and carbon dioxide content, depending on the motive regime and the nature of the work performed.

As a criterion for evaluating any gaseous environment we must use both indices of the general physiological state of various functional systems of the body and criteria characterizing general reactivity.

We have been faced with the problem of finding the relation between the gaseous environment in a sealed compartment, the motive regime, the nature of operator activity and resistance to acute hypoxia. To do this brief and prolonged (to 20 days) experiments were conducted with human participation as well as tests on animals.

Tests on animals. The studies were conducted on laboratory male rats weighing 170-190 g, placed in sealed chambers specially equipped for prolonged occupancy in which was created and maintained a set artificial atmosphere, containing oxygen and carbon dioxide in high and low concentrations (pO_2 85-

680 mm. Hg; pCO_2 45.6-76 mm. Hg) with normal and reduced total pressure. The content of oxygen and carbon dioxide in the occupied chamber was controlled by gas analyzer equipment. Temperature in the chamber varied within limits of 21±2°, relative humidity was 60-70%. /48

Resistance of animals to acute hypoxia ("reserve time" at altitude of 12,000 m during ascent in a pressure chamber at a rate of 25 m/s) was determined in the initial state, daily while in the artificial atmosphere and for 7-10 days after its replacement by ordinary ground atmosphere (aftereffect period). The test was stopped when convulsions and agonized breathing appeared. In determining altitude resistance in rats, heart and respiration rates were recorded and rectal temperature measured. In a separate series of tests, biochemical studies were also conducted.

Studies in man. Prolonged experiments were made on healthy males aged from 23 to 35 years. Resistance to acute hypoxia ("reserve time" at altitude of 7500 m) was determined in a GBK-63 pressure chamber before and after a prolonged period in an artificial atmosphere with elevated (pO_2 300 and 400 mm. Hg) and reduced (pO_2 105 mm. Hg) content of oxygen, increased content of carbon dioxide (pCO_2 to 45 mm. Hg) and with the combined effect of hypoxia and hypercapnia with normal and reduced total pressure in the chamber.

In determining "reserve time", continuous recordings were made of the subjects' heart and respiration rates and oxygen saturation of the blood, the gas composition of alveolar air was determined and continuous observation was maintained. The test was ended when the subjects developed menacing symptoms of acute oxygen starvation (presyncope, local convulsions, inadequate reactions, significant disturbances in the rhythm of respiration and heart activity, etc.).

A special series of tests on 14 pilots and 13 flight cadets investigated the preservation of consciousness and working capacity as well as the nature and quality of operator activity (sensory motor coordination process) while

breathing a hypoxic gas mixture, corresponding in oxygen content to an altitude of 7500 m.

To evaluate operator activity and sensory motor coordination were recorded the number of movements of the elevator and rudder-bar and their values, the time of a complex reaction to light stimuli and the quality of the reaction (number of errors) and the number of deviations of a point of light from the middle of a screen.

In carrying out this work, 60 studies were conducted on 41 subjects and 750 tests made on animals.

In our early research it was shown that tolerance of acute hypoxia depends on the gas composition and temperature regime of a sealed cabin [9, 10].

As we have already indicated, from a practical point of view it was also important to find out how the general reactivity of the body and resistance to extreme effects are changed after various times spent in an artificial atmosphere.

Studies with prolonged (to 10 days) stays of animals in an atmosphere containing high concentrations of oxygen (680 mm. Hg) have shown that in the first days, "reserve time" at altitude of 12,000 m increases in comparison with the initial data and later, on the other hand, as the result of the toxic effect of oxygen it is sharply reduced. An especially pronounced reduction of /49
altitude resistance was observed immediately after the atmosphere with a high oxygen content was replaced by an ordinary atmospheric environment. "Reserve time" at an altitude of 12,000 m here was reduced to 1.5 min., while in the original state it averaged 11.5 min.

In an atmosphere with pO_2 of 405 mm. Hg, hypoxic disturbances developed more slowly and were restored more rapidly after replacement of the hyperoxic environment with an ordinary atmosphere.

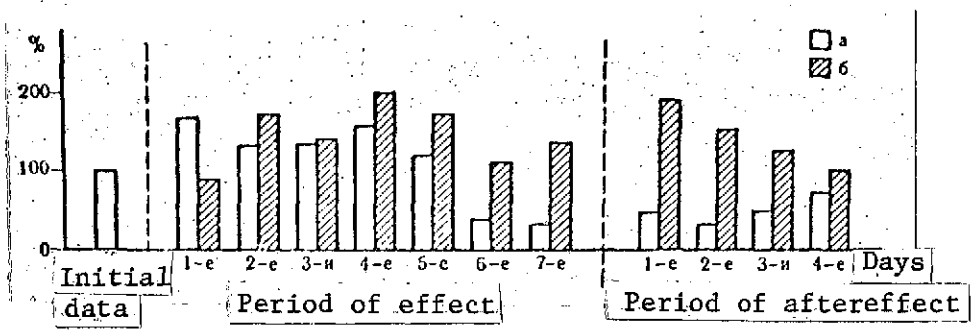


Figure 1. Survival time of animals (in percentages) at "altitude" of 12,000 m after various times spent in a hypercapnic environment (a — pCO_2 45.6 mm. Hg) and in a hypoxic-hypercapnic gaseous environment (b — pO_2 85; pCO_2 45.6 mm. Hg) with normal and reduced pressure in a pressure chamber.

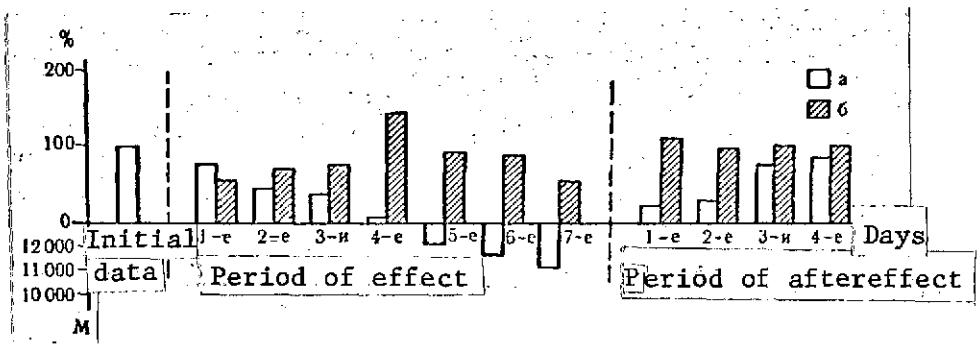


Figure 2. Survival time of animals (in percentages) at "altitude" of 12,000 m after various times spent in a hypercapnic environment (a — pCO_2 76 mm. Hg) and hypoxic-hypercapnic gaseous environment (b — pO_2 85; pCO_2 76 mm Hg) with normal and reduced pressure in a pressure chamber.

In a hypercapnic gaseous environment (pCO_2 45.6 mm. Hg) altitude resistance in animals increased in the first 3-4 days, but later, beginning with the 5th day, was progressively reduced in comparison with initial data (Fig. 1).

Increase of pCO_2 to 76 mm. Hg caused an even greater reduction of resistance to acute hypoxia. By the end of the first day in a hypercapnic environment, "reserve time" at altitude of 12,000 m was reduced an average of 25%, and on the 5-6th day animals in none of the tests were able to reach an "altitude" of 12,000 m (Fig. 2).

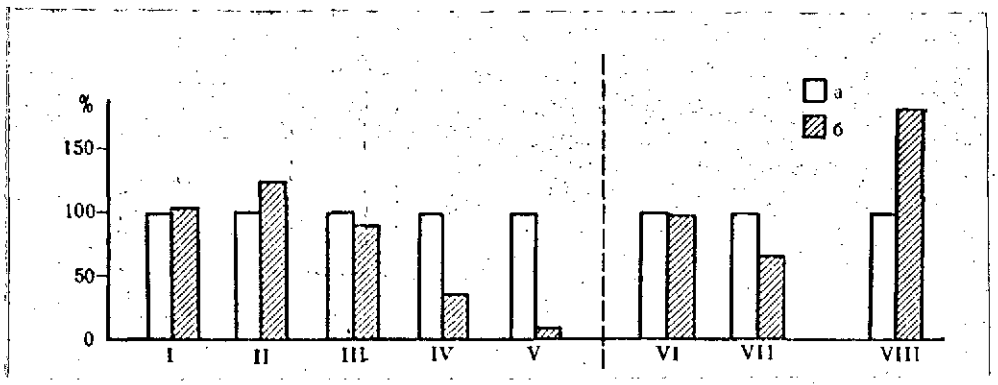


Figure 3. Reserve time at altitude of 7500 m after a prolonged stay by subjects in a hypercapnic environment (in percentages).

а — reserve time before effect of CO_2; b — reserve time after effect of CO_2

	mm Hg				mm Hg		
	P	pO_2	pCO_2		P	pO_2	pCO_2
	760	159	2	V	760	159	45,6
II	760	159	22,8	VI	405	159	22,8
III	760	159	30,4	VII	405	159	38
IV	760	159	38	VIII	510	159	22,8

With a prolonged (to 7 days) stay in a hypoxic gaseous environment (pO_2 85 mm. Hg) with total pressure reduced to 405 mm. Hg in the chamber, altitude resistance of the animals also decreased, although less significantly than in tests with pure hypercapnia. Evidently, staying under such conditions for 5-7 days is not enough to achieve stable adaptation, increasing the resistance of the body to acute hypoxia. Studies conducted at various times of mountain acclimatization at an altitude of 3200 m showed that resistance to acute hypoxia increased only after 15-17 days in the mountains and functional systems were stabilized even later — in 23-26 days [1, 7].

In a hypoxic-hypercapnic atmosphere (pO_2 85; pCO_2 45.6-76 mm. Hg) an increase in resistance to acute hypoxia was observed in animals, especially with pCO_2 of 45.6 mm. Hg (Fig. 3), which agrees with numerous literature data

[61, 68, 80, 83, 85].

Studies have shown, therefore, that during a prolonged stay of animals in an artificial atmosphere, changes in tolerance of acute hypoxia were due not only to the pO_2 and pCO_2 in the environment, but also to the length of the stay.

Having revealed the dependence of changes in altitude resistance on the gas content of the environment and the length of stay in animal tests, we felt it important to conduct studies under analogous conditions with human participation. These studies established that the "reserve time" at an altitude of 7500 m after 10-14 days spent by the subjects in a hyperoxic gaseous environment with pO_2 of 300 and 400 mm. Hg at ground pressure was reduced in comparison with initial data. Under such conditions, resistance to heavy physical loads was reduced and the amplitude of pulse reaction during work on a bicycle dynamometer (1200 kg-m/min for 5 minutes) increased sharply.

It is interesting to note that after prolonged altitude acclimatization in the Central Tien Shan mountains, a sharp increase was observed in altitude resistance: "reserve time" at altitude of 7500 m doubled in the subjects, but altitude ceiling increased an average of 30% [5, 7]. /51

After spending a prolonged time in an artificial atmosphere with pO_2 of 125 mm. Hg, resistance of the body to acute hypoxia also increased. If "reserve time" at altitude of 7500 m was 38 minutes in subject G. and 33 minutes in subject O. before the creation of a hypoxic gaseous environment in the chamber, after 6 days in this environment it was increased in both to 60 minutes, i.e., it was nearly doubled.

Although tolerance of acute hypoxia increased in a gaseous environment with pO_2 of 105 mm. Hg, it was less than with pO_2 of 125 mm. Hg.

There is basis for assuming that normalization of hypoxic disturbances, development of adaptive reactions and stabilization of physiological indices

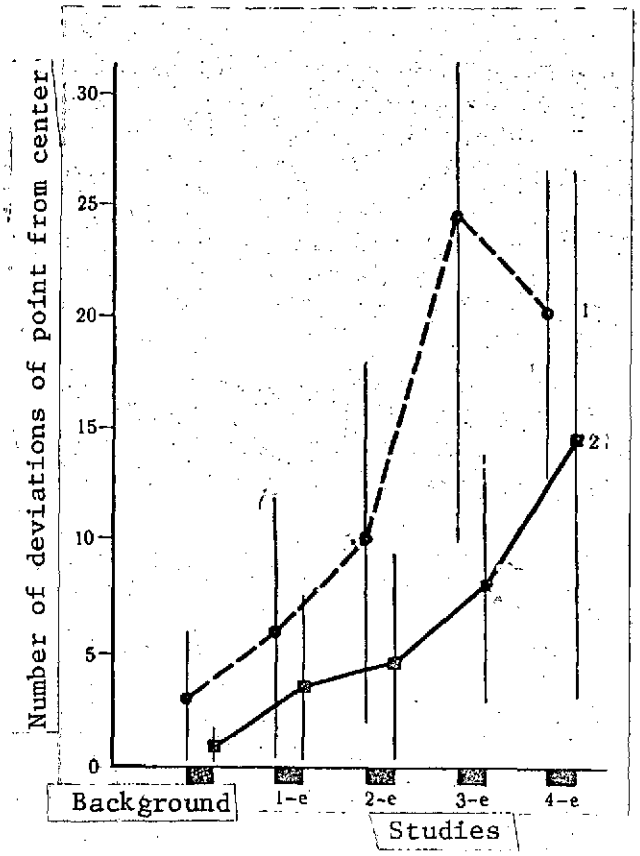


Figure 4. The effect of acute hypoxia on the quality of compensatory tracking process
1 — pilots; 2 — students.

in proportion to the reduction of pO_2 in inspired air to 105 mm. Hg and below requires significantly more time than with pO_2 of 125 mm. Hg.

After 25 days of mountain acclimatization at an altitude of 3200 m (pO_2 of 105 mm. Hg), altitude resistance in the same subjects was much greater than after 6 days in a pressure chamber with an analogous gaseous environment. Better tolerance of acute hypoxia after prolonged adaptation to high mountains is also indicated by such objective indices as oxygen saturation of the blood, EKG data, external respiration, etc.

The results we obtained agree with data of other authors who have shown /52
that after preliminary adaptation to hypoxia, resistance of the body increases not only to insufficiency of oxygen in inspired air, but also to the effect of a number of other extreme factors [7, 16, 78].

In a hypercapnic gaseous environment, tolerance of acute hypoxia also depended on the concentration of carbon dioxide and the length of its effect. With pCO_2 of 22.8-30.4 mm. Hg, "reserve time" in the subjects increased (Fig. 4). Further increase in the concentration of carbon dioxide (pCO_2 of 38-45.6 mm. Hg) caused a reduction in altitude resistance, as in such case, because of the toxic effect of carbon dioxide, fine interactions between oxygen and carbon dioxide were disturbed and the body began to experience a double load from the combined effect of hypoxemia and hypercapnia.

With the combined effect of hypoxia and hypercapnia (pO_2 125; pCO_2 11 mm. Hg) "reserve time" in all subjects increased 2-10 times in comparison with initial data. At the same time, with an increase in the concentration of carbon dioxide and reduction in the concentration of oxygen (pO_2 105; pCO_2 22.8 mm. Hg) in the environment, altitude resistance is practically unchanged.

Studies have, therefore, shown that hypercapnia in combination with hypoxia has a positive effect on the body only up to a certain level.

Having established the dependence of altitude resistance on the gas composition of the artificial atmosphere in a series of experiments on pilots and flight cadets, we studied the effect of acute hypoxia not only on the preservation of consciousness and working ability, but also on the performance of operator activity.

Results of studies showed that while breathing the hypoxic mixture, reserve time for students averaged 383±55 sec., for pilots it was 400±20 sec., i.e., it was approximately equal (Table 1). As acute hypoxia increased, a gradual reduction in mental and physical working capacity was observed in

TABLE 1

"RESERVE TIME" IN SUBJECTS BREATHING A HYPOXIC GAS MIXTURE CORRESPONDING TO ITS OXYGEN CONTENT AT ALTITUDE OF 7500 M

Students		Pilots		Students		Pilots	
Initials	"Reserve time" sec.	Initials	"Reserve time" sec.	Initials	"Reserve time" sec.	Initials	"Reserve time sec.
G. K.	390	Ts. M.	312	K. V.	298	S. T.	302
A. A.	535	O. Z.	307	F. A.	306	K. I.	410
P. Z.	405	Z. G.	540	A. Z.	373	R. R.	371
B. I.	373	S. T.	386	T. I.	417	Z. G.	417
V. V.	352	K. R.	356	T. N.	446	P. A.	458
P. N.	401	B. R.	465	S. Z.	419		
L. B.	379	Z. V.	365				
G. A.	506	R. I.	290	Average data	400		388

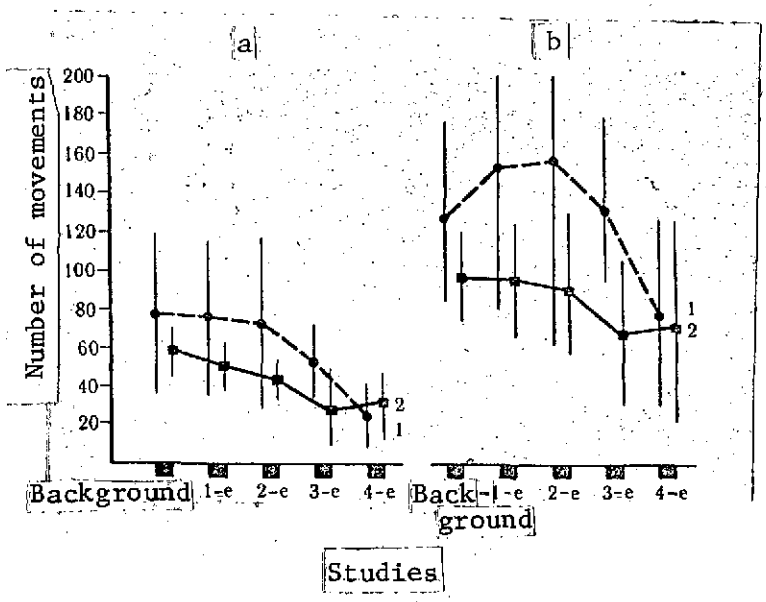


Figure 5. The effect of acute hypoxia on the number of compensatory movements of the elevator (a) and rudder bar (b)

1 — pilots; 2 — students.

/53

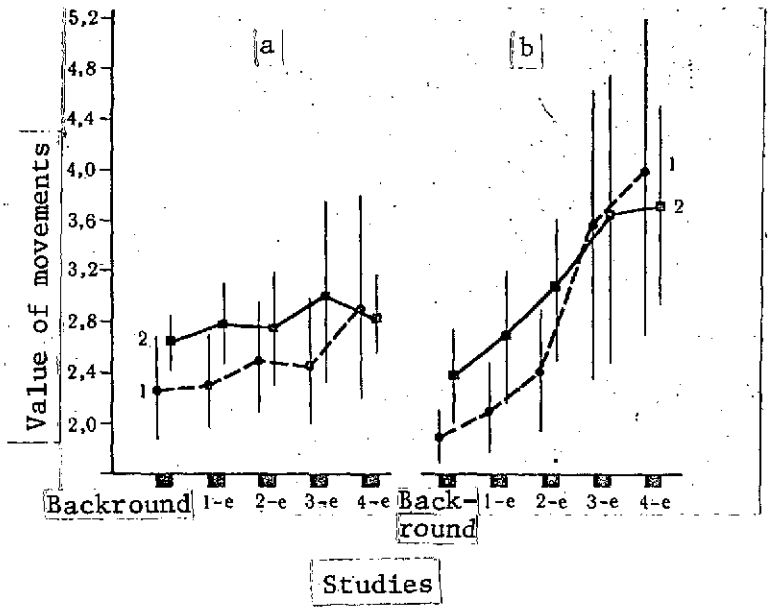


Figure 6. The effect of acute hypoxia on the number of compensatory movements of the elevator (a) and rudder bar (b)
1 — pilots; 2 — students.

the subjects. It appeared in a change in the number and value of corrective movements of the controls (elevators and control bars) (Fig. 5, 6). The value and number of movements of the elevator were, therefore, statistically reliably reduced. Approximately the same changes were observed in work with the rudder bar, with the only difference that in pilots, during the first 3 minutes of the effect of acute hypoxia the number of movements was greater than in students which is, evidently, because flying skills were stronger in pilots. Later (beginning with the 4th minute) the number of movements decreased sharply in all subjects. This indicates, evidently, that until a certain degree of hypoxia, habits developed during professional experience do not undergo significant changes. With further increase of hypoxia, individual differences in tolerance of acute oxygen starvation are erased.

The average number of deviations of a point of light from the center of a screen in the 4th minute of the test increased from 2.14 to 15.6 times in students and from 3.61 to 20.66 times in pilots, i.e. approximately sevenfold.

The time of a complex reaction to light stimuli changed insignificantly. At the same time the number of erroneous reactions was greatly increased (in students from 0.14 to 1.40 and in pilots from 0.15 to 1.83). /54

It is interesting that disturbances of these indices of sensory motor coordination reached maximum levels at the 4-5th minute of breathing the hypoxic mixture. At the same time the most significant changes in the cardio-vascular system and respiration were also detected; these changes were more pronounced in students (Table 2).

With relatively small changes in physiological parameters under hypoxic conditions, significant disturbances of sensory motor coordination and psychomotor function are observed. Disturbances of psychomotor coordination became substantial with oxygen saturation of the blood below 75%. It was shown that the first signs of disturbances of human mental activity under the effect of acute hypoxia appear with average reduction of arterial oxygen saturation under 64%, and syncope sets in with reduction to 56-62% [102, 103, 105]. However, in our studies we were not able to establish a clear relation between the severity of the hypoxic state, the degree of oxygen saturation of the blood and the quality of operator activity. Previously it was shown that with the same degree of hypoxia, oxygen saturation of the blood in different persons varied within quite wide limits [3, 59].

In joint studies conducted by the USSR and Czechoslovakia, it was shown that with the effect of acute oxygen starvation, consciousness undergoes a number of successive complicated changes. First disturbed is the ability to perceive indifferent signals and those with no great biological or social significance. Losses of consciousness appear at 65% oxygen saturation of the blood or less [6].

Analysis of experimental material, therefore, as well as our previous studies, indicate that resistance of acute hypoxia depends not only on conditions of the environment and motive regime, but also on the kind of operator /55

TABLE 2

CHANGES IN HEART RATE, ARTERIAL PRESSURE AND OXYGEN SATURATION OF THE BLOOD OF FLIGHT PERSONNEL UNDER CONDITIONS OF ACUTE HYPOXIA

Time of effect	Students			Pilots		
	Heart rate	Arterial pressure, mm. Hg	Oxygen saturation of blood,%	Heart rate	Arterial pressure, mm. Hg.	Oxygen saturation of blood,%
Initial level	74.2	120/85	96.0	76.0	125/75	96.0
1 minute	100.0	140/80	93.0	102.6	135/80	94.0
1 min 30 sec	106.2	---	90.6	103.4	---	93.2
2 minutes	108.8	150/80	87.0	105.0	150/80	89.2
2 min 30 sec	112.6	---	84.6	105.8	---	87.2
3 minutes	115.0	155/70	81.4	108.2	150/80	83.3
3 min 30 sec	120.3	---	80.3	108.8	---	82.4
4 minutes	132.0	160/70	76.2	114.4	150/75	81.4
4 min 30 sec	136.6	---	76.0	122.3	---	78.0
5 minutes	144.7	165/65	73.8	130.0	155/75	74.6
5 min 30 sec	146.2	---	72.2	136.4	---	73.4
6 minutes	153.8	155/60	69.8	137.0	155/70	73.2
6 min 30 sec	144.4	---	68.9	140.2	---	71.0

and professional activity.

N75 14410

On the Characteristics of Caloric Nystagmus in Healthy Persons

As is known, determining the limits of normal and pathology is impossible without definition of the limits of physiological fluctuations in studied functions of the body, taking into account sex and age. We studied electronystagmograms recorded in caloric tests (according to Fitzgerald and Hallpike) on 107 healthy males aged from 20 to 40 years. In all subjects, anamnesis revealed no diseases of the ears or vestibular disturbances. Special examination showed no changes in ENT organs; hearing was normal (audiometry).

Subsequent calorization of the ears according to this method was conducted in a darkened room with the head of the subject in optimum position for

stimulation of horizontal semicircular canals. Intervals between effects were 5 minutes. Nystagmus was recorded on a Hungarian four-channel nystagmograph with the subject's eyes closed; additional loads for physiological intensification of nystagmus were not applied. Registration conditions were always constant: electrodes were fixed in the outer corners of the eyes, skin-electrode resistance fluctuated between 5000-8000 Om; the time constant of the instrument was 0.3 sec., tape speed was 15 mm/sec. Calibration was carried out with standard deflection of the eyes 10° to the side, amplitude of the calibrated signal was 10 mm, the filter was 7.5 Hz. The length of the nystagmic reaction was determined from completion of stimulation of the labyrinth until appearance of the reversion phase of nystagmus or by termination of nystagmus. The frequency, amplitude and rate of the slow phase (s.p.r.) were studied for 20 seconds during the culmination phase of nystagmus, when all characteristics reach maximum levels and preserve relative stability [79].

Analysis of the studies was directed toward distribution characteristics of activity, frequency, amplitude and s.p.r. of nystagmus in the test group, as well as toward reflector asymmetry of the labyrinths and directional preponderance of the reaction. In addition, variants of the nystagmic reaction were determined according to the method of Bodo, who in previous studies distinguished the five following variants of nystagmic reactions: 1) weak reaction (the frequency of nystagmus less than 2 Hz, amplitude less than 2°); 2) medium reaction (normal according to Bodo)(uniform frequency of nystagmus within limits of 2-3 Hz, amplitude 2-10°); 3) strong reaction (frequency of nystagmus exceeds 3 Hz, amplitude was above 10°); 4) uneven reaction (frequency and amplitude of nystagmus during the culmination period changed and differences in frequency were over 2 Hz, the differences in amplitude were over 5°); 5) nystagmic reaction interrupted by a pause (interval between individual cycles was more than 2 sec.).

Quantitative characteristics of the asymmetry of reflex activity of /56
labyrinths and directional preponderance of the reaction were calculated according to the formulas of Jongkees and Philipszoon [104] with results com-

puted in percentages of total activity. We give these formulas:

I. Asymmetry of reflex activity of labyrinths:

$$\frac{(1+3)-(2+4)}{1+2+3+4} \times 100 = \ldots \%,$$

where 1 — results of calorization of the left labyrinth with 30° water; 2 — calorization of the right labyrinth with 30° water; 3 — calorization of the "left" labyrinth with 44° water; 4 — calorization of the "right" labyrinth with 44° water.

A plus sign in the result indicates greater activity of the left labyrinth and a minus sign means greater activity of the right labyrinth.

II. Directional preponderance of the reaction was determined according to the formula:

$$\frac{(1+4)-(2+3)}{1+2+3+4} \times 100 = \ldots \%.$$

Symbols are the same, but a plus sign in the result indicates a preponderance of reactions to the right, a minus sign — preponderance of left reactions.

In testing asymmetry, calculations were made according to the following parameters of nystagmus: s.p.r., amplitude, frequency and duration. Numerical material from parameter studies was subjected to statistical analysis by the Student method. As the result of statistical analysis of data, limits of physiological fluctuations of these parameters of nystagmus were determined in the group of subjects. Their indices were reliable.

Average indices correspond to literature data [99, 112, 124]. Calorization with hot water (40°), differing 7° from body temperature analogously to the cold water (30°), was accompanied by a less pronounced reaction in all parameters of nystagmus, although averages and their variability for the majority of indices of nystagmus with cold and hot calorization are very close. This is also verified by data on characteristics of nystagmus reactions ac-

cording to Bodo.

As can be seen from Table 3, a weak nystagmic reaction with cold water calorization (30°) was observed in 35.5-36.5% of cases; analogous indices with hot water calorization (44°) were 45-42.2%. A strong reaction to cold

TABLE 3

CHARACTERISTICS OF NYSTAGMIC REACTION ACCORDING TO BODO (IN PERCENTAGES OF TOTAL NUMBER OF SUBJECTS)

Type of reaction	30° water		44° water	
	left ear	right ear	left ear	right ear
Weak	35.5	36.5	45.0	42.2
Medium (normal)	21.5	21.5	22.4	23.4
Strong	9.3	9.3	5.5	5.5
Uneven	26.2	28.0	22.4	23.4
Nystagmus with pause	7.5	4.7	4.7	5.5
Total	100	100	100	100

calorization exceeded that with hot calorization more than 2 times. /57

Symmetry of labyrinth function according to individual parameters of nystagmus was detected in only 6.5-14.9% of the subjects. Greater activity of the left labyrinth was observed slightly more often. Thus, greater activity of the left labyrinth (according to s.p.r.) was noted in 47.6% and in the right in 45.8% of subjects. There were also analogous relations with respect to other parameters of nystagmus.

A certain directional preponderance of nystagmic reactions was also typical of healthy persons in the tested age group. Absence of this phenomenon according to individual parameters of nystagmus was established in only 3.6-13% of subjects.

These materials demonstrate more frequent preponderance of nystagmic reactions to the right in healthy persons. The frequency of this regularity according to ordinary nystagmus parameters was observed in 64-73.8% of subjects.

Quantitative characteristics of greater reflex activity of the right or left labyrinth in healthy individuals do not significantly differ in any nystagmic parameters. Thus, the average s.p.r. of the left labyrinth is 25±5.1% and of the right 23±3.7%, but amplitude is 28 and 27% respectively. At the same time quantitative characteristics of the directional preponderance of the reaction significantly differ in all tested parameters. Average indices of preponderant reactions to the right exceed corresponding characteristics of preponderant reactions to the left almost 2-3 times. Thus, preponderant reactions to the right, according to the frequency of nystagmus, was 39±4.7%, to the left 17±3.8%, and according to amplitude — 41±3.9% and 23±5% respectively.

TABLE 4

QUANTITATIVE CHARACTERISTICS OF THE ACTIVITY OF LABYRINTHS OF HEALTHY PERSONS (IN PERCENTAGES OF TOTAL ACTIVITY)

	Characteristics of activity	Statistical index	Parameters of nystagmus			
			duration	frequency	amplitude	s.p.r.
Activity of labyrinths	S > D	M ± m	16.0±3.3*	15.0±4.7*	28.0±3.5*	25.0±5.1*
		n	49	49		51
	D > S	M ± m	17.8±4.2*	14.0±4.0*	27.0±4.8*	23.0±3.7
		n	12	47	49	49
	D > S	M ± m	0	0	0	0
		n	16	11	8	6
Directional preponderance of reactions	D > S	M ± m	35.7±4.4*	38.0±4.7*	41.0±3.9*	39.0±1.2*
		n	73	69	79	76
	S > D	M ± m	12.4±6.8**	17.0±3.8*	23.0±5.0*	23.0±4.7*
		n	20	31	23	27
	D = S	M ± m	0	0	0	0
		n	15	7	5	4

Legends: s.p.r — slow phase rate; D — right labyrinth; S — left labyrinth; M — arithmetical mean; m — mean error;
* — with P > 0.001; ** — with P > 0.01.

Therefore, preponderance of reactions to the right is observed not only more often, but also quantitative characteristics of directional preponderance (d. p.) of the nystagmic reaction to the right are greater (Table 4). /58

Indices of the preponderance of reactions to the right correspond approximately with the upper limit of d.p. presented in the literature, observed in healthy persons, but indices of preponderance of reactions to the left is close to the lower limit. The significant preponderance of reactions to the right which we noted in all tested parameters of nystagmus is, evidently, due to greater activity of the left central vestibular formations. Such preponderance of reactions to the right is, therefore, analogous to "right handedness," [as is] the greater activity of the right vocal chord observed in the majority of healthy persons [73]. All these phenomena can be due to a certain preponderance of functional activity of structures of the left hemisphere of the brain.

The correctness of this assumption (d.p. characteristics of various direction) is verified by the definite participation in the reaction of the labyrinth of central links in the vestibular system, in connection with which quantitative characteristics of the preponderance of reactions to the right exceeded those to the left 2-3 times, but differences in reflex activity of the right and left labyrinths, according to average data, were insignificant.

Thus, these studies showed the possibility of asymmetry of reflex nystagmus in healthy persons. Reliable quantitative characteristics of the preponderance of functional activity of one labyrinth or the other were established, as well as the degree of directional preponderance of the nystagmic reaction in healthy persons.

Results of studies can be used clinically, in the practice of vestibular professional selection and in physiological tests. Mechanisms of asymmetry of reflex activity of labyrinths and especially directional preponderance of the nystagmic reaction require more precise definition. In particular, the theory of Cowthorne et al., [96] on the significance of general neuro-humoral and hemodynamic shifts as one cause of asymmetry of nystagmus must be confirmed by corresponding research.

N75 14411

Gas Exchange and the Coagulation System of the Blood during the Effect on the Body of High Concentrations of Oxygen and Carbon Dioxide

Interest in the effect on the body of high concentrations of oxygen and carbon dioxide has increased, not only as the result of physiological justification of parameters of the gaseous environment in hermetically sealed closed compartments, but also in connection with the use of these gases in clinical practice, aeronautics and cosmonautics. It must be noted that information existing in the literature on permissible concentrations of oxygen and carbon dioxide for maintaining normal life is extremely contradictory.

At the present time the problem of maximum permissible concentration of oxygen and carbon dioxide in the environmental atmosphere is being raised anew, especially with their prolonged effect on the living organism, as studies in recent years have shown that the time factor of the effect per se is of independent biological significance and introduces a number of features to the effect on the body of an altered gaseous environment [1-5]. /59

We studied the effect of high concentrations of oxygen and carbon dioxide on gas exchange, the coagulation system of the blood and tolerance of several extreme factors — acute hypoxia, acceleration, heavy physical loads. For this purpose, in addition to numerous brief studies, two series of long experiments were conducted.

In series I, subjects were kept for 25 days in a small sealed chamber (8 m^3), for 10-14 of these days in an atmosphere with pO_2 of 300-400 mm. Hg. In series II of the experiments, the length of occupancy in the chamber was 10-12 days, of which 5-6 days were in a hypercapnic gaseous environment (pCO_2 23-45 mm. Hg). A total of 35 brief and 5 long experiments were conducted with participation of 25 subjects.

Changes in gas exchange with prolonged stay in a hyperoxic and hypercapnic environment. As in breathing in an atmosphere with a high content of oxygen the greatest changes are noted in lung tissue, it was important to study the dynamics of changes in gas exchange during increased partial pressure of oxygen.

The tests showed that during a prolonged (to 14 days) period in an atmosphere with pO_2 of 300 mm. Hg, external respiration functions (rate and depth, minute volume of air, vital capacity of the lungs, inspiratory and expiratory

TABLE 5

INDICES OF EXTERNAL RESPIRATION IN SUBJECTS DURING OBSERVATION (AVERAGE DATA BY PERIODS[1])

Index	Subject M			Subject N		
	period			period		
	initial	hyperoxia	after-effect	initial	hyperoxia	after-effect
Respiration rate per minute	11	9	11	7	9	8
Minute volume of air:						
actual, l/min	5.05	7.08	5.3	4.2	5.9	5.2
% of normal	71.0	99.5	75.5	70.1	99.5	88.0
Consumption of oxygen:						
actual, ml/min	234	279	238	215	260	215
% of normal	85.4	98	84.8	90.5	109.5	91.5
Release of carbon dioxide, ml/min	178	231	197	166	217	177
Vital capacity of lung:						
actual, ml	4600	4338	4413	5250	4750	5012
% of normal	102	97	99	124	112	119
Expiratory reserve volume, ml	1050	865	1010	1888	1220	1390
Maximum ventilation of lungs:						
actual, l	100	97.5	97	112	91.5	111
% of normal	115	112	112	152	124	150

[1]In initial period — from three tests; in the periods of hyperoxia and aftereffect, from four tests.

reserve volumes) did not undergo serious changes. As the result of the increased content of oxygen in breathed air its consumption increased 15-28%. Partial pressure of oxygen in alveolar air p_AO_2 increased from 100-110 to 210- /60
255 mm. Hg and p_ACO_2 from 37-39 to 42-44 mm. Hg. Along with this an increase was observed in maximum ventilation of the lungs (6-14% during the experiment and 9-16% in the aftereffect period).

In a hyperoxic gaseous environment with pO_2 of 400 mm. Hg, changes in external respiration were greater (Table 5).

As seen from Table 5, the respiration rate, vital capacity of the lungs and expiratory reserve volume were reduced. Basal metabolism during the period the subjects spent in the hyperoxic environment increased in comparison with initial data 18-20%, pulmonary ventilation 28-30% and consumption of oxygen an average of 13-19%. An increase in the consumption of oxygen was also observed in tests on animals kept for 21 days in a gaseous environment with pO_2 of 400 mm. Hg [3,4]. During the effect of hyperoxia (pO_2 400 mm. Hg) p_AO_2 in subjects increased from 95-105 to 300-340 mm. Hg and p_ACO_2 from 32-34 to 38-42 mm. Hg.

In a hypercapnic gaseous environment, the changes in respiration functions were slightly different and depended basically on the concentration of carbon dioxide in breathed air.

With pCO_2 of 23 mm. Hg the biomechanics of respiration did not change significantly and were within limits of usual physiological fluctuations. Staying in a hypercapnic gaseous environment with pCO_2 of 30 mm. Hg or higher increased the respiration rate in the subjects 3-9 per minute and the volume of air 2-3 times in relation to background data.

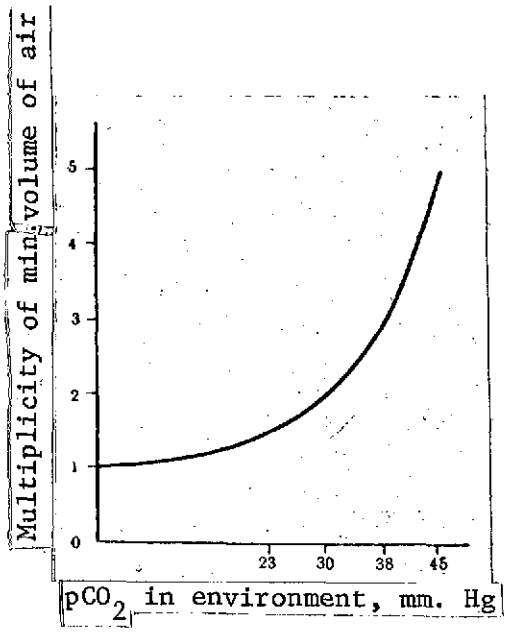
As the result of the increased respiration rate and respiratory volume, minute volume of air increased regularly. Under conditions of basal metabolism with pCO_2 of 23 mm. Hg it increased 1½ times, with pCO_2 of 30 mm. it doubled, with 38 mm. Hg it increased 3 times and with 45 mm. Hg 5 times in comparison

with initial data (Fig. 7).

Increased pulmonary ventilation was accompanied by additional work of the respiratory muscles which, in turn, led to increased consumption of oxygen by the body. Basal metabolism increased more in experiments the higher the concentration of carbon dioxide in breathed air. The first day the subjects were in the hypercapnic environment with pCO_2 of 23 mm. Hg, basal metabolism was increased 10-34% in comparison with initial data, with pCO_2 of 30 mm. Hg — 27-31% and with pCO_2 of 38 mm. Hg — 33-91%.

Besides the increased volume indices of respiration during hypercapnia, the qualitative composition of breathed and alveolar air changed. An increase in the concentration of carbon dioxide in breathed air caused an increase in the content of both carbon dioxide and oxygen in alveolar air. With pCO_2 in breathed air of 23, 30 and 38 mm. Hg, p_ACO_2 increased 3-5, 8-10 and 8-11 mm. Hg respectively and with p_AO_2 — 18-20, 28-30 and 25-32 mm. Hg. When pCO_2 in breathed air was increased to 45 mm. Hg, the carbon dioxide gradient between breathed and alveolar air decreased from 37 mm. Hg with normal composition of surrounding air to 8 mm. Hg (p_AO_2 was 53 mm. Hg); the oxygen gradient decreased from 55 to 24 mm. Hg (p_AO_2 reached 126 mm. Hg) (Fig. 8).

These changes in external respiration are verified by study of blood gases and acid-base equilibrium. As a result, in the hypercapnic gaseous environment there was a shift in the active reaction of the blood toward acidosis (reduction of pH, increase of p_ACO_2, p_AO_2 and true bicarbonates). With pCO_2 /61
of 23 mm. Hg the shift in the active reaction of the blood was regulatory in origin, but with further increase in the concentration of carbon dioxide in breathed air metabolic disturbances appeared sooner, the higher pCO_2 in the chamber. Complete renal compensation of respiratory acidosis in proportion to a 5-day stay in a hypercapnic gaseous environment with pCO_2 under 23 mm. Hg gives basis for assuming that the human body can adapt to such conditions. One of the most important mechanisms of adaptation is the reabsorption of HCO_3^-



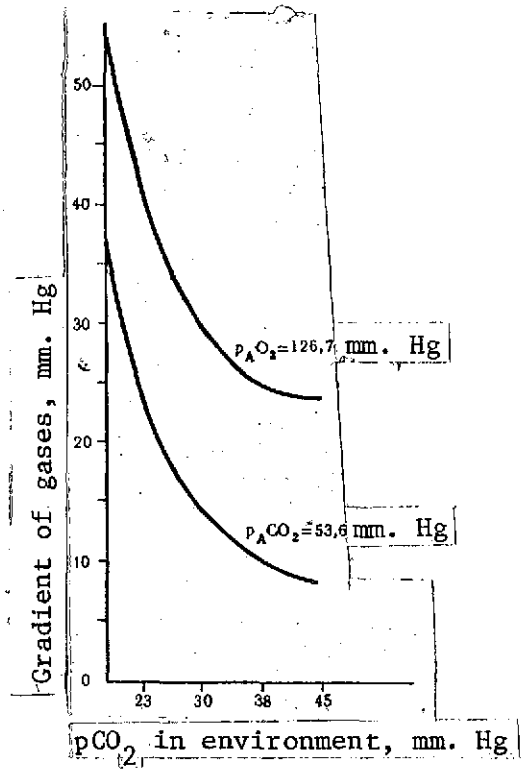


Figure 7. Pulmonary ventilation in man during basal metabolism with various pCO_2 levels in the environment.

Figure 8. p_ACO_2 and p_AO_2 gradients with various pCO_2 levels in the environment.

ions by kidneys and the accumulation of bicarbonates of the blood [86].

Signs of physiological adaptation in man during a 5-day stay in a gaseous environment with pCO_2 of 23-38 mm. Hg under conditions of rest are a decrease in pulmonary ventilation and the level of basal metabolism, as well as a reduction in excitability of the respiratory center to carbon dioxide, which is indicated by the gradual increase in breath holding time during the phase of maximum expiration and inspiration in proportion to the stay in a hypercapnic environment and by the reduction of breathing during night sleep during the aftereffect period.

To obtain information on accomodative reactions of the external respiratory system of man in a hypercapnic gaseous environment, gas exchange was studied not only under basal metabolism conditions, but also while performing a 10-minute physical test on a bicycle dynamometer at 500 kg-m/min. This /62 showed that while in a normal gaseous environment ventilation at rest was 5-10 liters/min., during work of 500 kg-m/min. it rose in subjects to 15-32 1/min., which is a 2.8-4-fold increase in this index. Oxygen consumption exceeded the initial level 2.2-5.9 times.

When the CO_2 content in breathed air was increased to 23-45 mm. Hg, pulmonary ventilation at rest rose correspondingly to 10-24 1/min., which exceeded minute volume of air at a state of rest in a normal gaseous environment 1.6-3.5 times. Heat production and oxygen consumption were not significantly increased.

Performance of a metered physical load under hypercapnic conditions caused a further increase in pulmonary ventilation, primarily because of the increased respiratory volume. With pCO_2 of 45 mm. Hg minute volume of air was 57 liters.

Thus, physical loads, increasing metabolic processes 3-4 times in relation to that at rest, cause greater changes in parameters of external respiration, the higher the concentration of carbon dioxide in breathed air.

The effect of hyperoxia and hypercapnia on the coagulation system of the blood. The mechanism of coagulation of the blood is one of the most typical examples of striving toward a state of equilibrium or dynamic regulation. Among vital processes we know of no other such system in which opposite changes appear with such biological regularity as in this process. Preservation of the fluid state of circulating blood is as vital a biological necessity as coagulation of that issuing from the blood stream. Such double function requires exceptional perfection of regulatory systems [118].

The literature contains only isolated information on the effect of an altered gaseous environment on coagulation and anticoagulation systems of the blood [24, 48, 60, 115, 116]. L. Palosh [118], on the basis of many years of studies, came to the conclusion that coagulability of the blood is regulated by oxidation processes and increase in the latter under hypoxic conditions reduces its coagulating activity. G. S. Kolot [48] noted an increase in coagulation time and reduction of thromboplastin activity in dogs after 15 minutes in an atmosphere of pure oxygen with pressure of 2.7 absolute atmospheres. In the opinion of G. I. Golovastova [24], an increase in oxygen content in breathed air causes a state of hypocoagulation in adult rabbits.

We could find no information in the literature available to us concerning changes in coagulability during the prolonged effect on the human body of an atmosphere with an increased content of oxygen and carbon dioxide. To solve this problem, the following indices were determined in subjects after various lengths of time in an altered gaseous environment: coagulability of the blood according to MacMagro, bleeding time according to Duke, amount of fibrinogen according to the Ruthberg method, fibrinolytic activity of the blood according to Koval'skiy, recalcification of plasma according to Howell, thromboelastography on a Hardtart instrument, and the morphological composition of the blood was studied.

Studies showed that the coagulation process undergoes important changes during many days effect on the human body of high concentrations of oxygen and carbon dioxide.

Hyperoxic gaseous environment. To discover the biological role of atmospheric oxygen, several series of brief tests were set up to study oxidation-reduction processes, to study regulation and functional unity of respiration, circulation and coagulation systems. It was assumed that hypoxia will cause increased coagulability of the blood and hyperoxia decreased. In tests in vitro it was established that under the effect of oxygen, thrombin is deactivated, while prothrombin shows significant resistance to it. This resistance /63

of prothrombin, as studies have shown [117], can be due to its activity in oxidized form, as its molecule contains four S-S groups. Although disulfide groups are reduced by cysteine, prothrombin loses its activity.

It is interesting that heparin in the composition prevents the oxidation of thrombin. This protective property of heparin also appears with the thermal deactivation of thrombin. Thus, at a temperature of 56° there is irreversible deactivation of thrombin; the presence of heparin in the environment prevents thermal deactivation and after 10 minutes at a temperature of 100° only minimal loss of activity of thrombin is detected.

The increased inclination toward coagulation and thrombosis, observed in patients with symptoms of decompensated hypoxemia, in our opinion, is due not only to the reduced biosynthesis of coagulation factors as the result of stagnation in the liver, but also to the direct effect of insufficient oxygen. It has been shown that local slowing of circulation and accompanying hypoxemia play an important role in the formation of a thrombus.

Analysis of data made it possible to formulate a theory of the functional unity of respiration, circulation and coagulation systems [115, 116, 117].

For a deeper study of the state of coagulation and anticoagulation systems of the blood, as we have already indicated, prolonged experiments were conducted on man. Remaining in a gaseous environment with pO_2 of 400 mm. Hg for 5 and especially 10 days lengthened reaction time (hr_1) and reduced maximum amplitude (Ma) and elasticity of the blood clot (E) on the thromboelastogram (Table 6).

According to literature data [43], maximum amplitude and elasticity of the blood clot are directly related to the concentration of fibrinogen in the blood, as well as the number and functional state of thrombocytes. The number of blood platelets and their functional activity also affect the formation of thromboplastin and thrombin, changing the reaction time (hr) on the

TABLE 6

THROMBOELASTOGRAM INDICES IN SUBJECTS DURING A PROLONGED STAY IN AN ATMOSPHERE WITH pO_2 OF 400 MM. HG

Index	Initial indices	Period of effect (days)		Aftereffect (days)		
		5	10	2	10	30
	Subject N					
Hr (min)	6.0	8.5	10.5	3.5	9.0	6.5
K (min)	4.0	3.5	4.0	1.0	3.5	2.0
Ma (min)	69.0	45.5	62.5	65.0	55.5	50.0
E	222	83	167	186	125	100
	Subject M					
Hr (min)	4.0	6.0	9.5	2.5	11.0	6.5
K (min)	2.0	2.0	4.5	0.5	3.5	2.0
Ma (min)	67.0	45.0	57.0	68.0	51.5	51.0
E	203	82	133	212	106	104

the thromboelastogram [51]. According to our material, the number of thrombocytes in subjects on the 5th day in a hyperoxic gaseous environment was reduced 47% in M. and 44% in N. On the 10th day the number of blood platelets in subject M was reduced even more and in subject N was slightly increased, nevertheless, remaining significantly reduced in comparison with initial data (see Table 6). /64

The hypocoagulation effect of prolonged hyperoxia indicated a relative increase in plasma recalcification time, which we noted in both subjects during the aftereffect period of a gaseous environment with pO_2 of 400 mm. Hg. If on the 5th day plasma recalcification time increased an average of 8%, by the 10th day of hyperoxia it had already increased 26% in comparison with initial data. As the time in the hyperoxic gas environment increased, the concentration of fibrinogen was reduced in both subjects. Thus, on the 10th day of hyperoxia the amount of fibrinogen was decreased 23% in one and 35% in the other subject in comparison with initial data.

The increased bleeding and coagulation time,which we noted in both subjects on the 5th and 10th day of the effect of a gaseous environment with pO_2 of 400 mm. Hg, verifies the hypocoagulation effect of prolonged hyperoxia. The slight reduction in fibrinolytic activity of the blood, which we observed in subjects during their stay in a hyperoxic gaseous environment, must be considered as an accomodative reaction of the body to reduction of coagulation activity of the blood.

Slightly different data were obtained when the subjects remained in a gaseous environment with pO_2 of 300 mm. Hg. The changed thromboelastograph indices greatly resembled the picture observed in an atmosphere with pO_2 of 400 mm. Hg, but the degree of their changes was significantly less. On the 11th day of hyperoxia, according to thromboelastograph data, an increase was observed in the coagulation capacity of the blood. Reaction time (hr.) and index K, characterizing the formation of thromboplastin, thrombin and fibrin, in both subjects decreased sharply. Maximum amplitude and elasticity of the blood clot increased significantly. In the opinion of some authors [116], such a phenomenon, in all probability, must be considered as the result of "supercompensating reactions," leading to deviations to the opposite side.

The tendency of the coagulation system to preserve a state of dynamic equilibrium is a basic biological law. Changes in any direction are the beginning of a series of subsequently developing compensatory reactions.

The number of thrombocytes on the 7th day in a gaseous environment with pO_2 of 300 mm. Hg is reduced only 15-21%. It is interesting that on the 11th day of hyperoxia when, according to thromboelastograph data, increased coagulation of the blood is clearly noted, the number of thrombocytes in both subjects is slightly increased.

Analogous changes are noted in recalcification of plasma and fibrinolytic activity (FLA) of the blood. If on the 4th and 7th days of the effect of hyperoxia an increase in plasma recalcification time was observed and a reduc-

tion of FLA of the blood was observed, on the 11th day plasma recalcification time distinctly decreased but fibrinolytic activity rose sharply. During this period the concentration of fibrinogen in the blood increased slightly in both subjects. In remaining days of the experiment the amount of fibrinogen, as /65 bleeding and coagulation time, did not change significantly.

It was extremely important to reveal the change in coagulation activity of the blood during relatively brief breathing of higher concentrations of oxygen.

TABLE 7

COAGULATION ACTIVITY OF THE BLOOD IN SUBJECTS DURING A PROLONGED STAY IN AN ATMOSPHERE WITH pO_2 OF 400 MM. HG

Index	Subject N					Subject M				
	Initial indices	Period of effect (days)		Aftereffect (days)		Initial indices	Period of effect (days)		Aftereffect (days)	
		5	9	2	6		5	9	2	6
Thrombocytes, thous.	369	206	149	182	280	245	130	133	245	262
Fibrinogen, g%	0.31	0.24	0.23	0.27	0.31	0.32	0.28	0.26	0.31	0.29
Recalcification time	103	112	126	96	112	127	138	156	100	128
Reticulocytes, %	4	-1	0	0	4	5	-2	0	1	5
Fibrinolytic activity, min.	168	176	202	146	178	152	160	182	134	162

Studies of coagulation activity of the blood, conducted before and after one day in an atmosphere with pO_2 of 715 mm. Hg (94% oxygen), showed that under such conditions a slowing down of coagulation processes is observed (Table 7).

Changes in indices characterizing the rate of coagulation, although not going beyond the bounds of physiological fluctuations, are, however, distinct, unidirectional and statistically reliable, with the exception of a reduction in fibrinolytic activity of the blood.

A study of the effect of brief (24 hour) hyperoxia (60% oxygen) on the coagulation activity of the blood showed that keeping a man in such an atmosphere for one day does not lead to marked changes in coagulation processes.

Undoubtedly, in the mechanisms of coagulation, the decisive role is played by oxidation-reduction processes, as a connection has been established between respiration and circulation, the mechanism of coagulation and biological oxidation [115, 116, 117, 118].

In a hypercapnic gaseous environment, changes in coagulation activity of the blood has the opposite character. In proportion to time spent in a hypercapnic gaseous environment with pCO_2 of 23, 30 and 38 mm. Hg, the number of thrombocytes increased 6-12%, erythrocytes 7-21% and leukocytes 6-17%. At the same time, an increase was noted in the concentration of fibrinogen in the blood of 38, 50 and 58% respectively. Maximum values of this index were reached with pCO_2 of 38 mm. Hg (Table 8).

Increase in the number of erythrocytes and the increased content of hemoglobin with simultaneous depression of erythropoeisis (reduction in the number of reticulocytes from 5 to 1-2%), especially in a gaseous environment with pCO_2 of 38 mm. Hg, is, evidently, connected with clotting of the blood because of loss of moisture by the body, due to hyperventilation and dilation of skin capillaries. Clotting of the blood and increase of its viscosity, as is known, /66 accelerate coagulation.

The reduced plasma recalcification time (24-38 sec.), the distinct reduction in bleeding time (19-63%) and coagulation time (12-24%) observed in an atmosphere with an increased carbon dioxide content indicate increased coagu-

TABLE 8

AVERAGE INDICES OF COAGULATION AND FIBRINOLYTIC ACTIVITY OF BLOOD IN SUBJECTS BEFORE AND AFTER EFFECT OF HYPEROXIA (94% OXYGEN)

Index	Before hyperoxia	After hyperoxia	P
Concentration of fibrinogen, mg%	300	260	0.001
Plasma recalcification, sec.	120	128	0.05
Fibrinolytic activity of the blood, min.	173	188	0.1
Bleeding time, sec.	150	176	0.02
Coagulation time, min.	9.7	10.8	0.02
Number of thrombocytes, thous.	333	267	0.001

lation capacity of the blood.

Results of a thromboelastographic study of coagulation processes of the blood indicate hypercoagulation, developing in the subjects in a hypercapnic gaseous environment. Thus, reaction time (hr) characterizing phases I and II of coagulation and the formation of thrombin on the 5th day in a gaseous environment with pCO_2 of 23-38 mm. Hg was reduced (8-26%). Index K characterizing the start of phase II of coagulation and the rate of blood-clot formation decreased 23-47% in comparison with initial data. Increased coagulation is also indicated by the increase in maximum amplitude (Ma) and elasticity of the blood clot (E), observed on thromboelastograms of both subjects during their stay in a hypercapnic gaseous environment.

Data obtained served as the basis for confirmation of hypercapnic mixtures in clinical practice.

In a patient suffering from hemophilia, L. Palosh attempted to stop severe renal hemorrhage which had lasted several weeks with carbon dioxide. For this purpose the patient was given a mixture of air and carbon dioxide to breathe. After intensive breathing of such a mixture the pathologically prolonged coagulation time, which reached 1 hour, after several minutes was first shortened to 2 minutes and then increased. With repeated breathings the

shortening of coagulation time continued to decrease and even greater lengthening. Finally, the shortening of coagulation time stopped altogether and the blood of the patient coagulated only after 17 hours. Hematuria remained unchanged until the end [118].

The increased number of formal elements, the increased concentration of fibrinogen, the compensatory increase on the second day and subsequent reduction of fibrinolytic activity of the blood, typical changes in thromboelastographic indices, the decreased recalcification time, shortening of bleeding and coagulation time all indicate that a hypercapnic gaseous environment causes /67 changes indicating increased coagulation capacity of the blood in man.

The gaseous environment and resistance of the body. As is known, general resistance of the body can change under the effect of a number of unfavorable environmental factors. Numerous studies have shown that a prolonged stay under conditions of an altered gaseous environment is accompanied by certain shifts in the functional state of various systems of the human body.

A study of accomodative reactions of the body under such conditions must ultimately be reduced to study of the reactivity of the body.

Experimental studies conducted by N. A. Agadzhanyan et al., [3, 9, 10] showed that changing the parameters of the gaseous environment in sealed inhabited compartments could cause a favorable change in the general reactivity of the body; in particular, resistance is increased to various extreme factors — acute hypoxia, acceleration, heavy physical loads, changes in temperature, etc. All this makes it possible to use such data as an additional criterion for physiological justification and selection of the most rational gaseous environment in sealed cabins.

In order to evaluate the general reactivity of the body resistance of the body to acute hypoxia, acceleration and heavy physical loads was determined before and after prolonged stay in an artificial atmosphere in a sealed chamber. Reserve time was determined in a pressure chamber at altitude of 7500 m.

Tolerance of laterally-directed "back-chest" acceleration was studied on a large-radius centrifuge. Subjects were exposed to accelerations of $+8G_x$. Tolerance of heavy physical loads was evaluated by performance on a bicycle dynamometer with a graduated load increase.

All the above noted studies were terminated upon development in the subjects of pronounced functional disturbances (presyncope, inadequate reactions, sharp reduction in working capacity, significant disturbances of respiratory rhythm and heart activity, etc.).

Results of changes in altitude resistance after prolonged occupancy of a pressure chamber with various parameters of the gaseous environment are given in Table 9. From these data it is evident that 10 days in the chamber with ground atmosphere parameters has no marked effect on tolerance of hypoxia. After a prolonged stay in a hyperoxic environment (pO_2 300-400 mm. Hg), reserve time at an altitude of 7500 m was reduced in subjects in comparison with initial data.

One of the most important mechanisms of adaptation in man to acute insufficiency of oxygen in transferring from breathing pure oxygen to breathing rarefied air at an "altitude" of 7500 m is the reaction of the respiratory system. After the oxygen mask is removed, respiratory rate increased in almost all subjects. Maximum increase was observed in the first 2-15 minutes breathing rarefied air. Breathing became forced with deep inhalation and exhalation of an average of 1 to 2 liters per breath. In some cases, on the other hand, there was a slight curtailment of breathing. Minute volume of air in the subjects was already increased 3-4 times in the second minute. In such cases when minute volume increased more than 5 times or when the reaction of increased pulmonary ventilation was absent in the subjects, as a rule, sharp /68
deterioration of general feeling was observed and the subject was given oxygen. In only 2 subjects did minute volume of air increase 5 to 7-fold at the very start of the test and later 15-fold (85-110 1/min) in a BTRS system; however, collapse was not observed in these persons. In the remaining 12 subjects, pulmon-

TABLE 9

COAGULATION ACTIVITY OF THE BLOOD WITH A PROLONGED STAY IN AN ATMOSPHERE WITH pCO_2 OF 23-40 MM. HG (AVERAGE DATA FROM TWO SUBJECTS)

Index	pCO_2 mm. Hg	Background	Effect		Aftereffect	
			2nd	5th	2nd	5th
			days		days	
Thrombocytes, thous.	23	320	333	342	314	270
	30	330	350	397	360	347
	38	308	284	332	307	307
Fibrinogen, mg%	23	0.23	0.30	0.32	0.27	0.26
	30	0.22	0.29	0.33	0.26	0.24
	38	0.22	0.28	0.33	0.27	0.25
Fibrinolytic activity min	23	175	157	132	157	167
	30	162	147	132	150	150
	38	165	127	230	205	180
Plasma recalcification, sec	23	126	116	114	124	126
	30	128	122	103	120	125
	38	128	110	97	103	117
Reticulocytes, %	23	4	2	2	3	4
	30	4	3	2	3	4
	38	5	2	1	2	4

ary ventilation was maintained at an average level of 18-25 l.

As we have already indicated, in determining reserve time at an "altitude" of 7500 m, stabilization — the establishment of a new resistant state of gas exchange between man and the gaseous environment — will be prolonged. After removal of the mask, p_AO_2 was high (180-220 mm. Hg) but p_ACO_2 was 28-39 mm. Hg. Calculations show that pO_2 in alveoli is reduced to 100 mm. Hg in approximately 30 sec. Then the air in the lungs becomes hypoxic, which is confirmed by the curve of oxygen saturation of hemoglobin. In 45 seconds after removal of the oxygen mask, i.e. with a 13-15 second lag in which the blood passing through the lungs can reach the ear, the curve begins to drop reliably. By the end of the 3rd minute, p_AO_2 was already 33-41 mm. Hg and p_ACO_2 20-27 mm. Hg. Later the level of alveolar oxygen and carbon dioxide was even slightly reduced, reaching 14-34 and 18-20 mm. Hg respectively by the end of an hour at an "al-

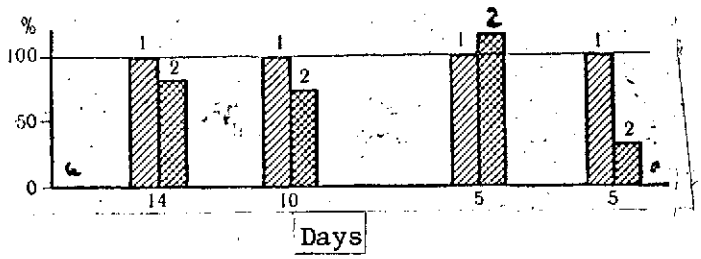


Figure 9. "Reserve time" at altitude of 7500 m after a prolonged stay in hypercapnic and hyperoxic gaseous environments (in percentages).

1 — "reserve time" before the effect of an altered gaseous environment; 2 — "reserve time" after the effect of an altered gaseous environment.

Environment	mm. Hg		
	p	pO_2	pCO_2
Hyperoxic			
14 days	760	305	2
10 days	760	403	2
Hypercapnic	760	159	22.8
5 days	760	159	22.8
5 days	760	159	38

titude" of 7500 m. After a prolonged stay in an altered gaseous environment, these values were practically unchanged (28-38 mm. Hg for oxygen and 10-20 mm. Hg for carbon dioxide).

The protracted character of the formation of a stable state of gas exchange appears especially clearly in the dynamics of changes in the respiratory coefficient which in the first 6 minutes significantly exceeded one; this is connected with the rapid release of oxygen and carbon dioxide from the body where there is some reserve of these gases in the blood and tissues. Later the respiratory coefficient was reduced to normal.

Because in the first minutes oxygen is not taken in by the body but released through the lungs into the atmosphere, its consumption, determined according to the Douglas-Holden method, is very weak and unstable. In our experiments it was from 25 to 406 mm. of oxygen per minute in the STPD system. However, by the 15th minute, when gas exchange begins to be established at a

relatively stable level, oxygen consumption already exceeds the initial level and is 300-600 ml. After a period in an altered gaseous environment, in individual cases we noted a tendency toward still greater increase of oxygen consumption by the body.

The change in altitude resistance after a prolonged stay in a hypercapnic gaseous environment depended on the level of pCO_2. An atmosphere with pCO_2 under 23 mm. Hg increased resistance to acute hypoxia in all subjects (Fig. 9). Further increase in the concentration of carbon dioxide caused a sharp reduction in altitude resistance and more serious changes in physiological reactions of the body. Thus, if in background studies before the creation of a hypercapnic atmosphere in the chamber "reserve time" at an altitude of 7500 m was 2 hours 30 minutes in subject N. and 7 minutes 35 seconds in subject A., after they were placed in an environment with pCO_2 of 38 mm. Hg it was 7 minutes 35 seconds and 4 minutes 25 seconds respectively. The most significant reduction in altitude resistance from 50 minutes to 3 minutes 25 seconds was observed with pCO_2 in the chamber of 43-45 mm. Hg.

Such a sharp reduction in the resistance of the body to acute hypoxia is connected with serious disturbances of functional systems of the body. Because of pronounced hyperventilation due to the specific effect of carbon dioxide on the respiratory center, on one hand, the expenditure of energy in /70
intensive work of respiratory muscles is increased and, on the other, against such a background of hypercapnia reactions appear more clearly [6, 8, 10].

Studies have shown that before a certain level, hypercapnia in conjunction with hypoxia has a stimulating effect on the body. The increased reaction of the respiratory and cardio-vascular systems improves oxygenation of the blood and prevents harmful aftereffects of hypocapnia. The increase of pCO_2 facilitates transfer of oxygen from blood to tissues and reduces the consumption of oxygen [61]. In addition, as the result of the local vaso-dilating effect of carbon dioxide the blood supply is increased and, therefore, also the supply of oxygen to such vital organs as the brain and heart [42, 74, 76]. The addition of carbon dioxide also increases oxygen saturation of the blood because

TABLE 10

"RESERVE TIME" AT AN ALTITUDE OF 7500 M BEFORE AND AFTER A PROLONGED PERIOD IN AN ALTERED GAS MEDIUM

Subjects	Normal gas environment		Hyperoxia				Hypercapnia					
	Control		pO_2 300 mm Hg		pO_2 400 mm Hg		pCO_2 22 mm. Hg		pCO_2 30 mm. Hg		pCO_2 38 mm. Hg	
	1	2	1	2	1	2	1	2	1	2	1	2
A-n	5 m 08 s	5 m 28 s									7 m 07 s	4 m 25 s
D-v	37 m 06 s	38 m 33 s					60 m 00 s	75 m 00 s	65 m 00 s	65 m 00 s		
Nef-v											96 m 00 s	68 m 00 s
N-v			7 m 40 s	4 m 40 s	6 m 51 s	6 m 10 s						
M-y					8 m 28 s	6 m 20 s						
Og-v									10 m 42 s	12 m 00 s		
Or-v							5 m 57 s	7 m 35 s			50 m* 00 s	3 m 25 s
R-v			7 m 37 s	4 m 40 s								

Note: m — minutes, s — seconds; 1 — "reserve time" before the effect of an altered gas environment; 2 — "reserve time" after the effect of an altered gas environment.

* Studies were conducted with pCO_2 of 45 mm. Hg (time in this gaseous environment was 36 hours).

of a shift in the oxyhemoglobin dissociation curve to the right [120].

Data on tolerance of acute hypoxia after a prolonged stay in a hypercapnic environment agree with results of centrifuge experiments conducted by N. A. Agadzhanyan, E. B. Shul'zhenko and others. It was found that man's resistance to laterally-directed accelerations of $+8G_x$ after being in an atmosphere with an increased content of carbon dioxide, as a rule, decreased (Table 10).

Analysis of results of the studies have shown that the basic mechanisms of these shifts are, evidently, hypercapnia and respiratory acidosis which, as

is known, also develops with a shorter stay in an atmosphere with a 3-5% concentration of carbon dioxide [35]. /71

Tolerance of heavy physical loads (1200 kg m/min for 5 min) after being in a hyperoxic environment with pO_2 of 300 mm. Hg was practically unchanged but with pO_2 of 400 mm. Hg was reduced. The amplitude of pulse reaction increased sharply. Systolic pressure had no such pronounced rise, i.e., adaptation of hemodynamics to a physical load traversed a less favorable path. Restoration of physiological indices after work was prolonged. A stable pulse condition was not established for 10 minutes, although before the experiment it was seen after 2-3 minutes of work.

In a hypercapnic gaseous environment, tolerance of metered physical loads changed depending on the concentration of carbon dioxide in breathed air. If with a pCO_2 of 23 mm. Hg working capacity was maintained at a sufficiently high level, with a further increase of pCO_2 it was decreased even in the first hours of hypercapnic conditions, especially significantly with pCO_2 over 38 mm. Hg.

Results of studies revealed that, with a prolonged stay in an altered gaseous environment, the character and degree of observed functional disburbances depend on the concentration of respiratory gases and the length of stay in an artificial atmosphere. By changing the composition of the atmosphere and bringing the gaseous environment into accordance with the work and rest regime and energy expenditures, the general reactivity of the body can be changed favorably and a sufficiently high level of working capacity be preserved.

Data indicate that in evaluating and standardizing parameters of the gaseous environment in inhabited sealed cabins, the maximum permissible concentration of oxygen, carbon dioxide and other gases must be evaluated not only according to data on changes in physiological reactions of the body during the course of a prolonged stay in an artificial atmosphere, but also by

results characterizing tolerance of extreme effects after various times in a particular gaseous environment. These results can be used for scientifically based use of oxygen and carbon dioxide in clinical practice and in the formation of a gaseous environment in inhabited sealed compartments.

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Correlation Changes in EEG, Conditioned and Behavioral Reactions with Various Degrees of Oxygen Insufficiency

At present there is extensive literature on the problem of hypoxia. Experimental data obtained in various countries have been generalized in monographs [15, 68, 78, 93, 101, 128]. However, many questions on this matter still remain unclear. It is especially important to study the time that working capacity and active consciousness are preserved under the effect of oxygen starvation and to establish the correlation between EEG changes and behavioral reactions under such conditions.

According to literature data, the dynamics of changes in bioelectric activity of the brain during acute hypoxia passes through three states: 1) predominance of rhythms of excitation at moderate altitudes; 2) predominance of /72
slow wave activity at altitudes above 9000 m; 3) depression of electric activity to complete "silence" at altitudes above 11,000 m [57, 59, 108, 113].

The first changes in conditioned reflexes are already observed at altitudes of 1000-4000 m and are expressed in an increase of conditioned reflexes, contraction of the latent period, increase of unconditioned salivation and uninhibited differentiation [11, 38, 111]. In man at these altitudes euphoria, exaltation and heightened motor activity are noted [78]. At altitudes of 4000-6000 m, hypnotic phase conditions develop in animals and at altitudes above 7000 m, food reflexes completely disappear but defense conditioned reflexes are preserved [3].

The authors explain these changes in conditioned reflex activity as an increase in the initial stages of hypoxia of the stimulating effect of the growing flow of impulses from chemo- and baroreceptors and later, the development of extreme protective inhibition as the result of insufficient oxygen supply to brain tissues.

In the majority of previous studies, recordings of the EEG and the conditioned reflex method were used separately during hypoxia and, therefore, no relation could be established between changes in electric activity of the brain and behavioral reactions. The establishment of such a relation, as well as revelation of a correlation between basic functional systems of the body under the effect of various levels of hypoxia, make it possible to investigate scientifically based means not only of predicting hypoxic disturbances, but also their prevention. The effect on the body of acute hypoxia was studied in man and rabbits.

Tests on animals were carried out under conditions of free movement with biopotentials recorded from the visual area of the cortex, lateral field of the hypothalamus, dorsal hippocampus and reticular formation of the mesencephalon. Nichrome electrodes with Bakelite insulation were inserted bipolarly 1-2 mm apart. Cork electrodes were inserted in bones of the skull.

A conditioned food-elicited reflex [21] was developed to light flashes (5 per second — positive; 2 per second — differentiation). A conditioned defense (avoidance) reflex [40] was developed to light flashes at a rate of 1 per second with parallel registration of the induced potential in the visual section of the cortex. The animals "ascended" in a pressure chamber to an altitude of 10,000 m at a rate of 25 m/sec. At the end of the experiment, histological studies were made of the site where the ends of the electrodes were placed.

Studies were also conducted on healthy males (aged from 20-30 years) who had passed medical flight boards and were pronounced fit for pressure chamber experiments and the effects of acute hypoxia without limitation. Changes in

physiological reactions of the body were studied while the subjects breathed pure nitrogen, helium or gas mixtures with a reduced content of oxygen, complex registration was made of the following physiological indices: EEG (8 leads), skin-galvanic reflex, motor reactions in solving arithmetic problems (letter test), EKG, rate and volume of respiration, oxygen saturation of the blood.

Motor reactions in solving arithmetic problems (subtracting by one from 1000) were recorded with the use of an electrocontact pencil. These reactions were noted on oscillograph tape simultaneously with recordings of the EEG, respiration and the EKG. /73

In the course of the studies, behavior of the subjects was observed continuously and after completion of the experiment their oral report of feelings and impressions of the test was recorded on magnetic tape. Results of physiological studies obtained in the course of the experiment were entered in an analog computer to model compensatory-adaptational regulatory processes of the body. A total of 45 subjects participated in 200 experiments studying the effect on the body of various levels of hypoxia, primarily the relation between EEG changes and behavioral reactions.

Under normal atmospheric pressure in all rabbits with developed conditioned food-elicited reflexes in response to a rhythmic light signal, bursts of synchronized fluctuations in potentials were observed on the EEG at a rate of 8-10 fluc/sec in all test leads as well as the assimilation of the rhythm of the light flashes in the visual zone of the cortex and lateral field of the hypothalamus. Such localization of the EEG reaction of impression in response to a conditioned food signal indicates stimulation of the visual area of the cortex (conditioned cortical center) and lateral field of the hypothalamus (unconditioned subcortical center). With the effect of a differentiated stimulus (light flashes at the rate of 2 per second), the EEG reaction of assimilation did not appear in any of these leads.

With the effect of moderate hypoxia (2000-3000 m), the character of EEG and conditioned reflex activity did not change significantly in comparison with initial data. However, during this period in the intervals between signals, bursts of biopotential fluctuations in the rhythm of the light conditioned stimulus were recorded in leads of the lateral field of the hypothalamus. At the same time, the number of intersignal reactions was increased. Presentation at such altitudes of a differentiated stimulus caused food-elicited movement of the animal (uninhibited) and on the EEG appeared a reaction of the assimilation of the rhythm of the inhibiting signal in leads of the lateral hypothalamus and visual cortex (Fig. 10). As the lateral hypothalamus is the unconditioned subcortical food center with cholinergic stimulation [41, 84, 92, 95, 123] and its artificial stimulation increases conditioned and unconditioned food reactions in animals, it can be assumed that this stimulation of anterior-lateral sections of the hypothalamus is responsible for facilitating conditioned food reflexes, shortening their latent periods and uninhibited differentiations observed at moderate altitudes.

In proportion to further increase of hypoxia at altitudes of 5000 m or more, the phenomenon of imposed rhythms in leads from the hypothalamus during intervals between signals disappeared; shortly after, the conditioned food reflex also faded. The application at such altitudes of a rhythmic light conditioned stimulus led to generalization of the EEG reaction of imposition. Presentation of a differentiated stimulus with such degrees of hypoxia also caused the generalized appearance of the EEG reaction of imposition: in the visual and temporal zones of the cortex, reticular formation of the mesencephalon and hypothalamus; in the posterior hypothalamus this reaction was better expressed than in the lateral section. Food-elicited movement was absent here (Fig. 11). This indicates increased stimulation of the nonspecific /74 activating system, particularly the posterior-medial hypothalamus and reticular formation of the mesencephalon, and loss of the biological significance of the conditioned food signal. Probably at altitudes of 5000-6000 m pulses leading from chemo- and baroreceptors cause the increased excitation of the subcortical nonspecific system, particularly its adrenergic mechanisms, which changes the food significance of the signal.

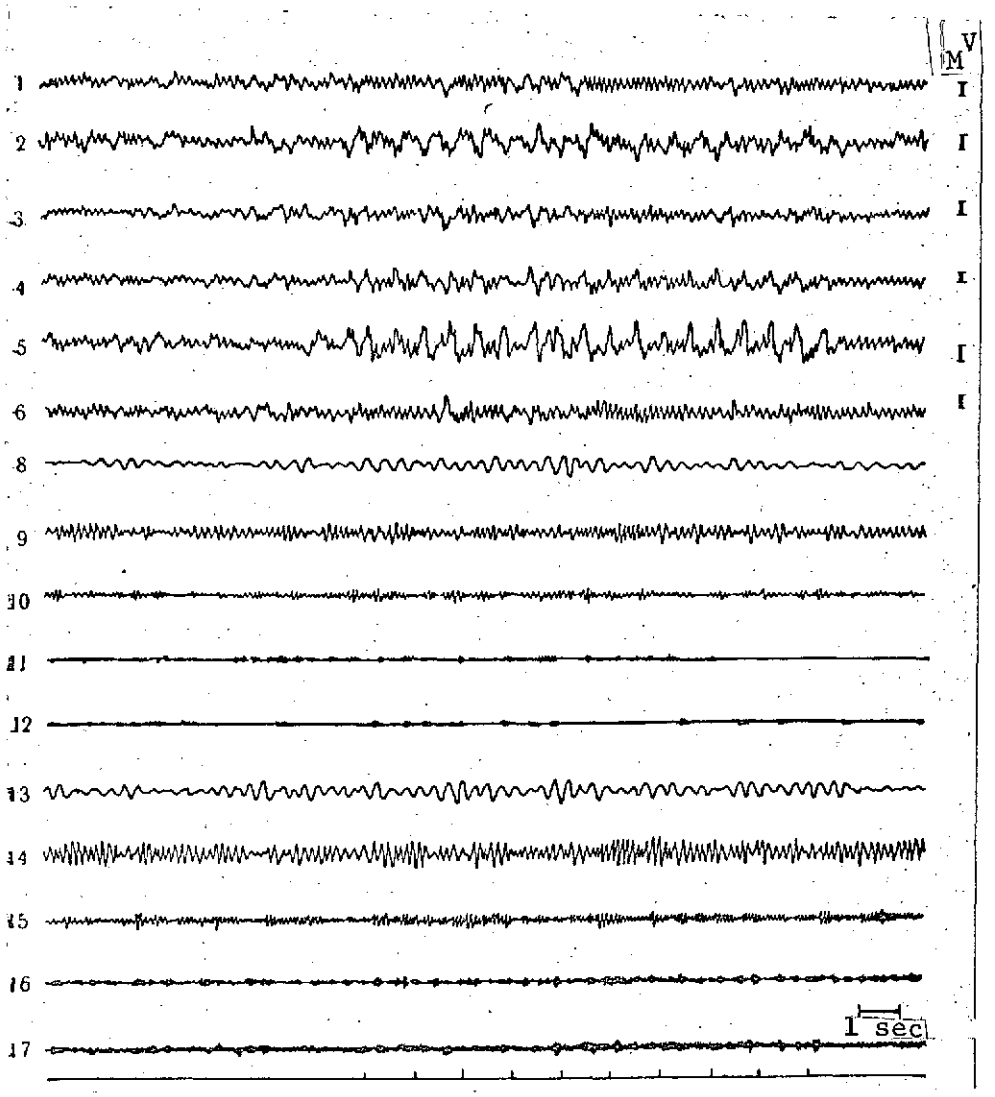

Figure 10. EEG changes with the presentation of an inhibiting differentiated segment at an altitude of 3500 m in rabbits.

1 — hippocampus; 2 — lateral field of the hypothalamus; 3, 4 — reticular formation of the mesencephalon; 5 — visual area of the cortex; 6 — posterior hypothalamus; 8-12 — frequency ranges of analyzer for EEG of the lateral field of the hypothalamus; 8 — delta; 9 — theta; 10 — alpha; 11 — beta-1; 12 — beta-1; 13-17 — frequency ranges of analyzer for EEG of visual section of the cortex; 13 — delta; 14 — theta; 15 — alpha; 16 — beta-1; 17 — beta-2. Lower line — conditioned reflex line.

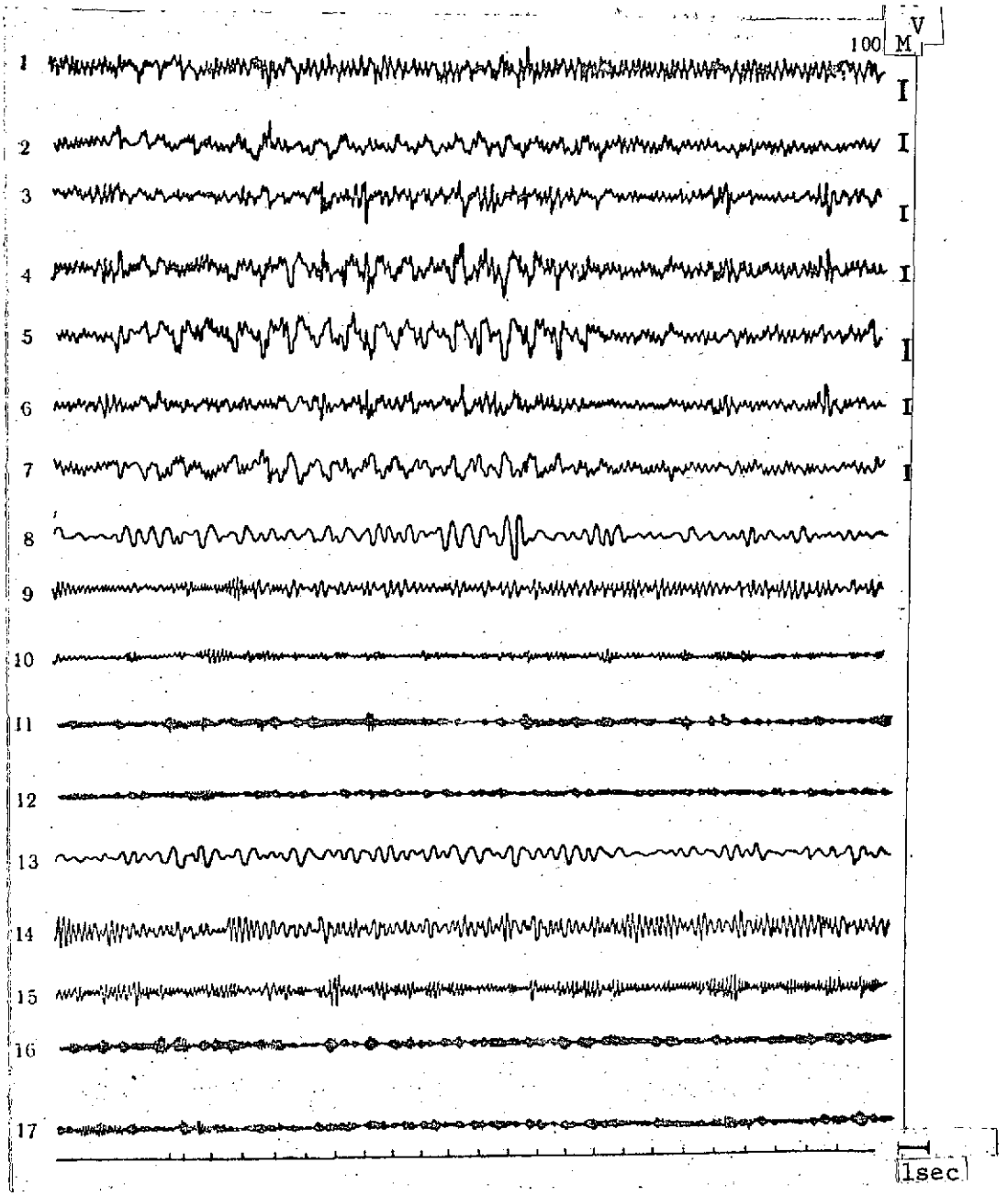

Figure 11. Changes in the EEG with the presentation of an inhibiting differentiated signal at "altitude" of 6000 m in a rabbit. Legend same as Fig. 10.

Changes in the EEG at altitudes of 6000-8000 m are characterized by the "transformation" of the imposition reaction in response to a rhythmic light signal in nonspecific structures of the brain and the preservation of its nor-

mal appearance in the visual area of the cortex. The conditioned food reflex is absent here. These changes, according to the concept of Vvedenskiy and Ukhtomskiy, can be explained as a symptom of parabiosis.

Finally, at altitudes of 8000-9000 m complete adynamia of animals is observed with the appearance on the EEG of slow high-amplitude waves which, as a rule, first arise in the cortex of the large hemispheres. Presentation at such altitudes of a conditioned rhythmic light signal causes a reaction of assimilation of the rhythm only in leads of the visual cortex, while in other leads it is absent. Probably, under the effect of hypoxia specific pathways of pulse transmission are less disturbed. Experimental facts give basis for assuming that at a given stage of hypoxia normal function of the nonspecific subcortical system is disturbed, which probably also causes the appearance of slow waves in the cortex of the brain and loss of consciousness. /76

During "descent," when theta-rhythms predominate on the EEG, the reaction of imposition is not recorded in any of the test leads; greater duration of the phase with no EEG reaction of assimilation was found to be directly dependent on the rate of descent. It can be assumed that during this period afferent impulsation from the rhythmic light signal is blocked because of external inhibition, developing with excitation of the central nervous system of a stress nature as the result of the aftereffect of hypoxia.

Placing animals in a conditioned reflex chamber immediately after "descent" and presentation of a rhythmic conditioned signal did not cause the imposition reaction in any of the test leads. Theta activity and bursts of synchronized fluctuations of biopotentials at a rate of 8-10 fluc/sec predominated on the EEG. There was no food-elicited movement. It can be assumed that at this time the block of afferent impulsation is continuing.

In 5-10 minutes after placing the rabbits in the conditioned reflex chamber, the reaction of assimilation of rhythm in response to a signal begins to be recorded, but only in leads of the visual area of the cortex and is ab-

sent in other test leads. Theta rhythm predominates on the EKG. Food-elicited movement is absent. Evidently, pulses from the signal reach the cortical conditioned center, but are not transmitted to the unconditioned center because of the development there of a different kind of excitation, in this case of a stress nature, as the result of which the conditioned signal is no longer of the former biological food significance. It can be assumed that in this phase specific pathways are already freed of stress excitation caused by hypoxia but the latter is still preserved in nonspecific structures of the reticular formation and hypothalamus. As a result, stress excitation, although it does not block the transmitted afferent volley of impulses along specific pathways, by forming a behavioral motivation reaction transforms the conditioned stimulus into a signal of a different biological significance. As the result, "efferent" pulses are blocked.

Finally, in 15-20 minutes after "descent", the food-elicited conditioned reflex appears in response to a signal, but at the same time on the EEG is recorded the reaction of assimilation of the rhythm in the visual area of the cortex and somewhat later in the lateral field of the hypothalamus. This indicates restoration of normal function of the central nervous system and disappearance of the stress condition.

Thus, the result of brief acute hypoxia is disappearance of the food conditioned reflex because of the development, primarily in structures of the non-specific activating system of the subcortex, of a state of stress excitation. In studying the dynamics of changes in the conditioned defense reflex /77 and parallel registration of the induced potential (IP) of the visual section of the cortex, the following was ascertained.

In rabbits before the conditioned defense reflex is developed and with extinction of the orientation reaction in response to a light stimulus (flashes), IP of the visual area of the cortex was expressed in a basic primary response (PR) with a latent period of 15-22 μsec. and amplitude of 55-105 μV. Later fluctuations of IP were unstable but the amplitude of the secondary response

(SR) was very insignificant and varied from 10 to 30 μV.

With a developed conditioned defense reflex, in response to the same signal IP underwent the following changes: some reduction of amplitude of PR and a distinct statistically reliable increase in the amplitude of the secondary positive deviation which fluctuated from 40 to 100 μV. With further combinations of the secondary response IP slightly decreased, but remained higher than with an indifferent light signal.

In "ascent" in a pressure chamber up to an altitude of 5000-6000 m no marked changes were observed in the defense conditioned reflex activity or components of IP in response to the signal. Beginning with these altitudes, a decrease in developed conditioned reflex movement is noted in test animals in response to the signal, but preservation of such with unconditioned electric pain stimulation. Here IP, as a rule, did not change, but in individual animals with separate presentations of conditioned stimulus, the amplitude of secondary positive deviation is increased under 100 μV on the average. It is very typical that the above noted changes usually appear immediately before appearance on the EEG of slow (delta) waves, which is often accompanied by an increase in the latent period of IP components.

With further "ascent" to altitudes of 7000-9000 m when delta activity is clearly recorded on the EEG, a disappearance of unconditioned defense reflexes was observed. Rabbits, as a rule, at this time lay unmoving but IP was changing: the latent period and amplitude of PR increased; variability increased; secondary positive deviation decreased or practically disappeared. In several tests at such altitudes IP practically disappeared.

Having revealed a correlation between EEG changes and behavioral reactions in tests on animals, we decided to find the depth and sequence of changes arising with the effect of acute hypoxia on man. It was found that in man, correlation relations between EEG shifts and behavioral reactions during acute hypoxia are expressed in a number of subsequently developing regular changes.

It is known that adaptation to hypoxia is a very complex and multifaceted process in which practically all organs and systems of the body are involved. The resulting function characterizing the effectiveness of compensatory-adaptational reactions of the body is the level of arterial oxygen saturation. The degree of arterial oxygen saturation is largely responsible for oxygen tension in tissues and, therefore, also for the normal course of oxidizing processes. It must be noted that oxygen saturation of the blood is determined not only by the degree, but also by the rate of increase of oxygen starvation.

The studies of N. A. Agadzhanyan et al., showed that the rate of increase /78
of hypoxia is of independent biological significance. Depending on it, oxygen saturation of the blood at a given altitude fluctuates from 48 to 76%. Individual differences appear especially clearly during a small rate of increase of hypoxia. In tests simulating disturbance of the vacuum seal of the cabin it was shown that the first signs of disturbances of mental activity appear with an average reduction of oxygen saturation of the blood to 64%; syncope appears at 56-62% [102, 103].

In our studies, hypoxia increased very rapidly. Time from the start of breathing pure nitrogen or helium until the appearance of severe functional disturbances averaged about 2 minutes with individual fluctuations of 5-8 seconds. Studies showed that the more rapidly oxygen starvation increases, the more individual differences are erased. These changes in physiological reactions of the body are typical of all tests.

Disturbances of higher nervous activity occur in man at the 50-55th second of breathing nitrogen or helium with oxygen saturation of the blood of 75-80% (Fig. 12) which coincided with registration of O-activity on the EEG. In the initial period of the appearance on the EEG of O-waves, significant changes were observed in the function of the central nervous system. Indifferent signals applied during this period were not, as a rule, perceived by the subjects. In proportion to the increase of hypoxia, and with oxygen saturation of the blood to 70% (75-80th second of breathing nitrogen or heli-

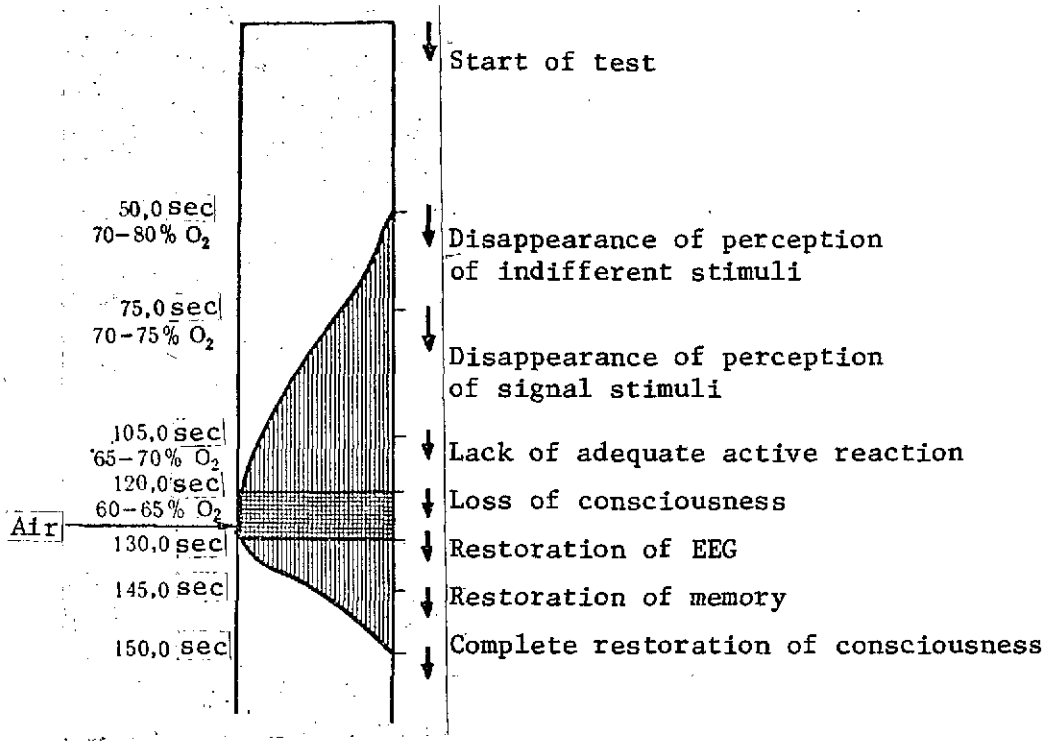

Figure 12. Changes in behavioral reactions in man during acute oxygen starvation.

Left — time after start of breathing pure nitrogen or helium and percent of oxygen saturation of the blood (oxyhemometry data); right — symptoms of functional disturbance. Shaded section — degree of expression of disturbances.

um), delta-waves appeared on the EEG (first singly, then grouped and, finally, /79
with deep hypoxia, delta-waves predominated on the EEG). The appearance of delta-waves accompanied regular changes in motive reaction as well as memory; the latter was expressed in errors committed in performing simple arithmetic problems and in symptoms of perseveration. In our opinion, these symptoms indicate functional dissociation between the cortex and subcortical (thalamic) sections. With predominance on the EEG of delta waves, reactions typical of altitude hypoxia often appeared which could arbitrarily be called a symptom of automatism. The subject continues to move his writing hand but is not able to write anything down. Later, with oxygen saturation of the blood to approximately 65% or less (115-125th second of breathing nitrogen and helium) consciousness is lost.

With the effect of acute oxygen starvation, consciousness undergoes a number of sequential and complex changes. First disturbed is the ability to perceive indifferent signals and signals having no great biological and social significance. The subject first losses the ability to perceive the surrounding situation as a whole with all details and indifferent stimuli; all attention — active consciousness — seems to be centered on the performance and perception of the main task of the experiment. Later this ability is also lost, as well as the sense of danger; the memory preserves only traces in the form of perception of propriocentric signals from body muscles. Then (in approximately 120 seconds) loss of consciousness occurs (see Fig. 12).

The loss of consciousness which arises with acute hypoxia (in our studies it lasted a total of 10-15 sec.) is of an adaptational character, as this disconnects structures performing no active information function. At a specific stage, those structures in the central nervous system continue to act which still provide function of certain vital regulatory processes (respiration, cardiac activity).

The capacity for adequate reaction to external signals and coordinated psychomotor activity after loss of consciousness (lasting 10-15 seconds) returns in man after 30 seconds, but the EEG picture is restored in 15 seconds.

Complex study of various functional systems shows that with repeated effects of hypoxia there are changes in the physiological reactions of the body directed toward better adaptation to sharply changing conditions in the gaseous environment. Thus, with the repeated production of acute oxygen insufficiency (breathing pure nitrogen after 62-second intervals), only in the first test was the classic picture of changes in body functions developed: hyperventilation, tachypnea, tachycardia. With subsequent effects, the reaction was distorted with regard to respiration (Fig. 13); shifts in ventilation were less pronounced and the tachycardia rate remained practically unchanged. We assume that such changes appearing with repeated hypoxia are connected with the development of adaptational mechanisms due to changes in functions of the central nervous system.

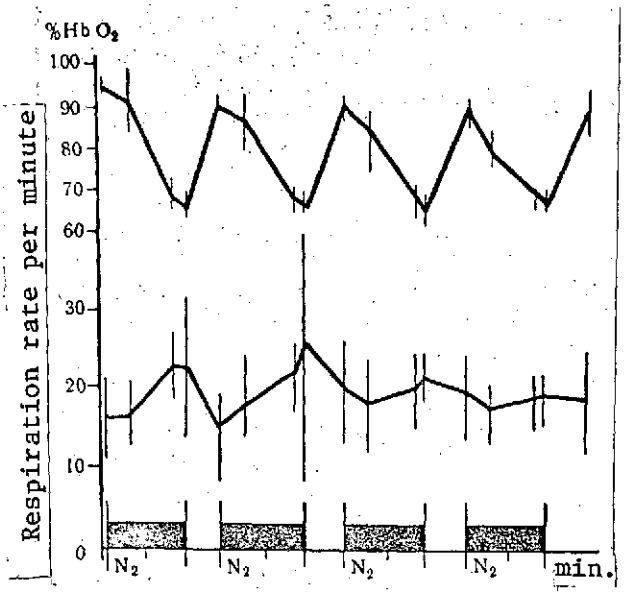

Figure 13. Changes in the dynamics of respiration in man with repeated effects of oxygen insufficiency.

Horizontally — marks for repeated sessions of breathing pure nitrogen. Vertically — (from top to bottom) — indices of oxymetry and respiration rate.

From these materials it follows that on the basis of the degree of oxygen saturation of the blood and EEG changes, those behavioral disturbances can be evaluated which can arise with various degrees of oxygen insufficiency. This is important to take into consideration in medical evaluation of the adequacy of responses of crew members to various signals during flights in modern aircraft.

Studies of Hypokinesia in Animals to Solve Urgent Problems of Space Biology and Medicine

One of the most important problems of space biology and medicine is the effect of weightlessness on the body. However, in ground experiments it is practically impossible to reproduce a state of weightlessness for prolonged periods. Therefore, in such tests various models are used which reproduce only individual symptoms of changes developing during true weightlessness.

In comparing results of experiments using ground models, it has been possible to obtain data of significant importance for understanding the general laws of change in the reactivity of the body during weightlessness. For this it is important to use all resources of comparative methods of research, using various models. This, in turn, will make it possible by comparative analysis of data to differentiate symptoms typical of only a certain kind of animal or a given model from general laws determining shifts in vital activities of the body and in functions of its most important systems under extreme conditions.

At the present time hypokinesia, myorelaxation and immersion of the body in water are being used in ground studies to simulate individual symptoms of /81 weightlessness.

Experiments with prolonged limitation of motor activity of man revealed a number of serious shifts in metabolic processes, the state of the cardiovascular system, the nervous system and support-motor apparatus [39, 50].

However, studies on man cannot completely solve the problem, as humane considerations severely limit the range of experimental effects applied to man in tests.

In this respect, experiments on animals present the researcher with unlimited possibilities. In such experiments various models of inactivation of

of the muscular system have been worked out [65], studies have been made of changes in the functional state of higher vegetative centers of the brain [89], changes in mediator and hormonal balance [87, 88], water-salt balance [49, 81], gas exchange [47, 75], protein equilibrium of muscles [22] and the structure of muscles [71, 72].

At the present time in the "Interkosmos" program, various experimental studies of hypokinesia are being conducted in tests on animals. Changes in hormonal and mediator balance during hypokinesia were studied in tests on rabbits by B. M. Fedorov and V. S. Nevstruyeva, in tests on rats by L. Tomashevska, G. Kats'yuba-Usilko and S. Kozlovskiy, in tests on birds by K. Boda. In tests on rats, studies in gas exchange and tissue respiration have been conducted by Ye. A. Kovalenko and E. S. Mailyan, the protein content of muscles by M. S. Gayevskaya and L. M. Kurkinaya, the structure of muscles by V. V. Portugalov and Ye. I. Il'ina-Kakuyeva, the function of skeletal muscles by V. S. Oganov and A. N. Potapov and the function of somatic nerves by S. Baran'skiy.

In this work we give some of the results of joint studies by scientists of socialist countries on the problem of hypokinesia and briefly generalize data on the effect of hypokinesia on: a) hormonal and mediator balance of the body; b) gas exchange and tissue respiration; c) protein content in skeletal muscles; d) the structure of skeletal muscles; e) the function of skeletal muscles.

The studies whose results are included in this work were conducted primarily on rabbits and rats. It is of great interest to compare the results of experiments conducted on these animals, as limitation of motor activity causes a sharp stress-reaction in rats while in rabbits the stress-reaction is very insignificant and brief.

Changes in mediator and hormonal balance. Test animals were placed in specially designed cages which severely limited motor activity. Rabbits

weighing 3-3.5 kg lost up to 0.6 kg during a month of hypokinesia. In connection with this, the walls of the cages were moved, making it possible to keep the animals under conditions of continuous hypokinesia. The animals were treated very carefully to avoid subjecting them to any traumatic effects. Test rabbits were killed in from 3 to 80 days by means of very rapid bleeding, cutting all the vessels in the neck area. The adrenalin and noradrenalin content was determined in adrenal glands, the myocardium and hypothalamus by the fluorometric method of Eiler as modified by Osinskaya. The catecholamine content was calculated in micrograms per gram dry weight of tissue. Under the effect of hypokinesia the content of catecholamines in various organs and tissues of the body changed markedly. /82

After 3 days in a state of hypokinesia, the adrenalin content rose in the adrenal glands of rabbits very insignificantly — on the average from 243 to 255 μg/g, but after 12 days it was reduced and even more sharply reduced after 1 month (to 91 μg/g).

The adrenalin content in the heart by the 12th day of the test did not differ from normal (0.17 μg/g) and was moderately reduced at the one month mark (0.12 μg/g).

A significant (2½ times) and completely reliable reduction in the level of catecholamines was noted in the adrenals of rabbits at later points of the tests. During hypokinesia noradrenalin in the myocardium and hypothalamus is reduced sooner.

Under the influence of 12-day hypokinesia, the noradrenalin content in the myocardium was sharply reduced — from 0.94 to 0.35 μg per g dry weight of tissue - and remained sharply reduced until the one month point. Under the effect of hypokinesia, the noradrenalin content was also sharply reduced in the hypothalamus (from 0.15 to 0.03-0.08 μg/g).

Hypokinesia causes distinct changes in the corticosteroid balance of the body. In rabbits kept in hypokinesia cages, the level of 11-oxycorticosteroids

in plasma of peripheral blood had been reduced to half (from 10 to 4.7 μg%) by the 12th day of the experiment and more than 3 times (to 2.9 μg%) by the 20th day ($P < 0.01$). In the 2nd month of hypokinesia these values remained reduced.

There is a great deal of interest in comparing the effect of hypokinesia on hormonal and mediator balance in tests on rabbits and rats. Naturally, under conditions of a severe stress-reaction, changes in hormonal-mediator balance were more complex in rats.

In experiments on rats it was found that the excretion of adrenalin with urine increases, beginning on the 5-10th day the animals are in the hypokinesia cages and is only established at a more or less stable level after the expiration of 20-30 days of the test. At the same time the excretion of noradrenalin with urine is reduced and after 15-25 days of hypokinesia is stabilized at a level significantly lower than the original.

It is typical that because of the pronounced stress-reaction in rats, changes in corticosteroid balance during hypokinesia were more complex than in rabbits.

In the first 30-35 days of the experiment, distinct intensification of the synthesis and secretion of corticosterone was observed; its content rose in the blood from 10.8±0.6 to 27.58±0.71 μg% and in the adrenals from 8.3±0.15 to 25.7±1.35 μg%. In the second month of hypokinesia in rats the content of corticosterone and ACTH in blood plasma was reduced to levels not significantly differing from those typical of control animals and in the third month of hypokinesia it was reduced much lower, being reduced by the 170th day of the test to almost half of normal.

Finally, in tests on birds it was established that hypokinesia sharply alters the reactivity of the body and its hormonal systems to stress effects.

Change in gas exchange and tissue respiration. In experiments conducted on rats it was noted that during hypokinesia, gas exchange increases, becoming especially pronounced by the 90-100th day of the experiment and at later points /83 of the test. Studies conducted using the polarographic method established that after 90-100 days of hypokinesia, the rate of oxygen consumption in individual sections of muscles increases rapidly, i.e. the intensity of regional gas exchange increases. However, gas tension in tissues does not deviate significantly from normal values.

Changes in tissue respiration, studied by the Warburg method, were expressed in depression of phosphorylating activity and often in a disturbance of the conjugation of oxidizing phosphorylation.

In the myocardium of rats assimilation of inorganic phosphorus on the 45th and 60th day of hypokinesia was reduced 41% ($P < 0.05$). The absorption of oxygen by the end of the 2nd month was also reliably less than in control animals, but only 17%. As a result the P/O coefficient on the 45th day was reliably reduced. By the 60th day of hypokinesia the reduction of the phosphorylation coefficient was less pronounced and after 100 days changes in this coefficient had disappeared. Thus, in heart muscle tissue during the 2nd month of hypokinesia a reduction was noted in the level of phosphorylation, a weakening of the intensity of respiration and an interruption of oxidizing phosphorylation.

In skeletal muscle the nature of changes was the same as in the myocardium, but deviations were expressed to a lesser degree. On the 45th day of hypokinesia bound inorganic phosphorous was reduced 35% ($P < 0.05$), on the 60th day — 34%. Oxygen consumption by muscle tissue throughout the experiment did not change. The P/O coefficient was reduced after 45 and 60 days. In tissue of skeletal muscle, therefore, there was a depression of phosphorylating activity during the 2nd month of hypokinesia.

These changes in heart and muscle tissue indicate that prolonged limitation of mobility of rats inhibited the process of phosphorylation, which de-

termines the energy potential of cells. Evidently, disturbance of the rate of macroerg generation is one of the causes of functional insufficiency of the cardio-vascular system and disturbances in physical working capacity.

Changes in the protein content of skeletal muscles and myocardium. There is special interest in changes in the protein content of skeletal muscles and the myocardium during hypokinesia. This section of the study was carried out in experiments on rats. Different varieties of tests were conducted, both changing the degree of limitation of mobility (less strict in box-cages and more strict limitation of mobility with the rats enclosed in a net) and diet, etc. For each form of the test, corresponding control experiments were conducted.

In order to sacrifice the animals and take tissue samples from them, the rats were either decapitated or immersed for 30 sec. head down in liquid nitrogen. The heart and tissues of the back legs were subjected to chilling but were not frozen.

The fractional composition of muscle proteins was determined by the Ivanov method with final determination of protein according to Lowry.

The experiments showed that placing the rats in narrow individual box-cages causes an increase in motor activity and muscular efforts during the first 30 days in an attempt to realize the "reflex of freedom." This led /84
(Table 11) to a reduction in the number of sarcoplasmic and an increase in the number of myofibrillar proteins.

Under such conditions of the test, rats became listless on only the 40-50th day of being in the box-cages. On the 60th day of limitation of mobility a reduction was noted in the protein content in intact muscle. At this point the number of sarcoplasmic proteins remained low but the number of myofibrillar proteins was not only less than that found on the 30th day of limitation of mobility, but also below control levels.

TABLE 11

THE EFFECT OF HYPOKINESIA (IN BOX-CAGES) ON THE PROTEIN CONTENT IN SKELETAL MUSCLES OF RATS (PERCENT OF CONTROL)

Index	Limitation of mobility					
	30 days			60 days		
	Total protein of intact tissue	Sarco-plasmic proteins	Myofib-rillar proteins	Total protein of intact tissue	Sarco-plasmic proteins	Myofib-rillar proteins
Triceps muscle of the calf	91.7	77.3	131.0	87.6	74.4	66.5
P	0.01	0.001	0.01	0.001	0.001	0.001
Quadriceps muscle of the calf	95.5	80.2	122.5	87.4	81.4	74.5
P	--	0.001	0.02	0.01	0.001	0.001

TABLE 12

AMOUNT OF PROTEIN (IN GRAM PERCENTS) IN VARIOUS PROTEIN FRACTIONS OF THIGH MUSCLES OF RATS DURING STRICT LIMITATION OF MOTOR ACTIVITY

State of animal	Statis-tical index	Total protein of intact tissue	Sarco-plasmic proteins	Acto-myosin (AM)	Fraction T	AM/T	Proteins of stroma
Control	M	17.7	4.09	4.85	2.42	2.05	2.07
	±m	0.66	0.09	0.29	0.24	0.19	0.15
	n	5	5	5	5	5	5
Hypokinesia 24th day	M	17.15	2.83	3.89	3.31	1.18	2.32
	±m	0.62	0.31	0.25	0.05	0.07	0.12
	n	5	5	5	5	5	5
	P_{init}		0.01	0.05	0.01	0.01	--

With other, stricter means of limiting the mobility of rats — by tying them in a net to a support — on the 24th day changes were already observed in the protein content of thigh muscles, typical of dystrophic processes (Table 12). The amount of sarcoplasmic proteins and actomyosin was reliably reduced, the protein content in fraction T increased and the ratio of actomyosin to fraction T (AM/T) decreased.

Strict limitation of mobility of the animals altered the protein content not only in skeletal muscles but also in the myocardium. Strict immobilization of the rats caused a reliable decrease in the content of total protein /85
and the amount of sarcoplasmic proteins in myocardium after 24 days. A tendency appeared toward reduction in the actomyosin content and the amount of protein in fraction T and stroma was reliably increased (Table 13).

TABLE 13

AMOUNT OF PROTEIN (IN GRAM PERCENTS) IN VARIOUS PROTEIN FRACTIONS OF THE MYOCARDIUM OF RATS DURING STRICT LIMITATION OF MOBILITY

Group	Statistical index	Total protein of intact tissue	Sarcoplasmic proteins	Actomyocin (AM)	Fraction T	AM/T	Proteins of stroma
Control	M	17.08	5.31	2.55	1.71	1.52	3.16
	±m	0.89	0.25	0.09	0.09	0.11	0.20
	n	5	5	5	5	5	5
Test	M	14.15	3.96	2.02	2.64	0.90	4.13
	±m	0.31	0.27	0.23	0.33	0.10	0.31
	n	5	5	4	5	5	5
	P_{init}	0.05	0.01	0.1	0.05	0.01	0.05

Thus, with stricter limitation of mobility, the "reflex of freedom" disappeared more quickly and dystrophic changes in skeletal muscles began to develop sooner. It is especially interesting that in mice kept in a state of hypokinesia, changes in proteins of the myocardium were of the same character as in skeletal muscles. These changes indicate the predominance of catabolic

processes over those of protein synthesis.

Structural and cytochemical changes in skeletal muscles. Studies were conducted on random bred male rats with an initial weight of 180-200 g, kept strictly immobilized. Our materials indicate that red (plantar and soleus) and mixed (peroneal and quadriceps) antigravity muscles of the rat react differently to limitation of function [71, 72].

At early points in the limitationoof mobility test, beginning on the first day, a sharp increase is noted in red muscles in the permeability of blood vessel walls, leading to the development of edema, and later — focal development of connective tissue. Quite early there is separation of some bundles of muscle fibers. Along the periphery, and sometimes also in the center of muscle fibers, there is a disturbance of lateral striation. The Z band first enlarges and then loses its continuity and is represented by irregular small lumps of various shape and size. The integrity of the contracting apparatus of the muscle fiber is disturbed. Protofibrils lose their continuity of course, breaking up into separate fragments.

On the 5-7th day of the test, besides being increased in size, muscle fibers are found in a state of decay. Besides the inclusion in the pathological process of muscle and connective tissue components of red muscle, changes are also observed in the nerve apparatus supplying the muscle. By this point, end-plates are found in which nerve fiber endings are completely or partially lysed.

By the end of the 2nd week of tests, muscle fibers are in a state of lumpy decay, vacuole and wax-like degeneration; foci of round cell infiltration are noted and newly formed connective tissue. A very typical change for red muscles (plantar and soleus) was the formation of "target-fibers." This was not encountered in mixed (fast) muscles — peroneal and quadriceps [72, 82]. With the appearance of myofibrillar ATPase, a reduction in enzyme activity was clearly visible in the middle of altered fibers.

On the 30th day, ATPase activity was not observed in middle sections of altered muscle. Therefore, contracting protein myosin was absent here. Complete disintegration of contractile structures was seen on electronograms in these areas. For this point of observation, the presence of a large number of hyperimpregnated nerve fiber endings is typical.

On the 60th day, there were many "target-shaped fibers" in specimens which, evidently, developed from "target fibers." "Target-shaped fibers" are characterized by lack of deposits of diformagan in the middle zone and a gradual increase of these deposits toward the periphery of fibers. In type I fibers, normally differing by high ATPase activity, there was an even reduction in the activity of the enzyme, which made identification of the type of muscle fibers in specimens difficult.

From the 45th day and later points in the tests, pictures of excessive growth of terminal branches of motor nerve fibers were typical. At later points of the experiments, an increase was noted in the mass of mature connective tissue (especially around vessels of the muscular type and capillaries), as well as atrophy and disintegration of muscle fibers. The nuclear apparatus of muscle fibers was reorganized, chains of nuclei appeared, often located in the middle of fibers.

Cytochemical study of enzymes indicates that during hypokinesia shifts occur in muscles in relation to the level of exchange processes in various metabolic cycles.

It is typical that changes occur and develop in structural elements of red (slow) muscles during hypokinesia, increasing during the course of the test. There is basis for assuming that the appearance of "target-fibers" and "target-shaped fibers" is directly connected with disturbances of muscle innervation. It must be pointed out that the degree of expression of structural and metabolic changes in red muscles in different individuals is not the same: in some individuals only isolated groups of fibers are found with these

changes, in others the process covers the entire muscle or a considerable part of it.

During hypokinesia, changes develop differently in mixed (fast) muscles — peroneal and quadriceps. In these muscles at early points of the test there are no symptoms of edema or connective tissue reaction. In neuro-muscular apparatus of muscles, no changes were observed at any periods of the test. At later times of the test (from the 30th day), centers of perivascular round cell infiltration were observed in peroneal and quadriceps muscles, primarily around arteries. On the 60th day, many vessels of the muscular type and capillaries had thick adventitia, consisting of densely packed fibers. Centers of mature connective tissue were found in peripheral sections of muscles, as well as individual muscle fibers or small groups of such in a state of vacuole degeneration and necrosis. There occurred atrophy of muscle fibers. This is especially clearly evident at points of the test after 30 days. These changes occurred against a background of metabolic changes in muscle fibers. /87

Thus, limitation of mobility is accompanied by the development of morphological and cytochemical changes in skeletal muscles. There is basis for assuming that in inactive muscle, or in muscle limited in function, primarily dystrophic and atrophic changes develop in muscular and neural elements which, in turn, cause a productive reaction in connective tissue which is secondary. Reduction of muscular activity is accompanied by stimulation of growth and differentiation of connective tissue. Besides sclerotic changes in muscle stroma, prolonged hypokinesia causes perivascular sclerosis. In turn, changes in vessels at later points of hypokinesia can be one of the most important factors underlying disturbance of nutrition of muscle tissue and further development of the pathological processes in muscles. Thus, hypokinesia causes a disturbance of the blood supply of muscle tissue, not only in connection with general changes in hemodynamics, but also because of the reorganization of the vascular wall. There is basis for assuming that reduction of the tone of the vascular wall of blood vessels in man during prolonged bed rest [64] is connected with the development of a "sleeve" of collagen fibers around muscular

type vessels and capillaries.

Changes in the function of skeletal muscles. Studies in this section were conducted on male Wistar rats initially weighing 170-180 g. Test animals were kept in narrow plexiglass chambers whose volume was increased in proportion to the growth of the animals. Control rats were kept in groups of 10 in cages measuring 450 x 450 x 370 mm. In the course of 4 months after the start of the test, control and test animals were alternately removed for acute tests. The preparation and test were conducted under nembutal narcosis.

The state of neuromuscular specimens of the following muscles were studied: soleus (SM), plantar (PM) and extensor digitorum longus (EDL). For this purpose the sciatic nerve and distal tendons of these muscles were extracted and severed. To provide an isometric regime, the knee and ankle joints were immobilized by wires inserted in bulges of the thigh bone. Extension of muscles was optimum. The recording method has been described in the work of Yu. M. Bazzhin et al. [14]. The nerve was stimulated by platinum electrodes from an EST-10 stimulator with square pulses lasting 0.5 msec. The following functional characteristics of muscles were determined: threshold force of the stimulus, frequency of superposition and fused tetanus, tension development time and half-life time of a single response, development time of half tension and half-life time of a tetanic response with stimulation at a rate of 100 pulses per second for 1 second, maximum amplitude of single responses and amplitude of maximum tetanus. Every month of the experiment 10 control and 10 test animals were examined. The work gives average (M) ± error of average (m) (Fig. 14).

Evaluation of the excitability of muscles was based on determination of thresholds of indirect stimulation. Unreliable changes were noted in the form of increased excitability of all muscles in the 1st month of hypokinesia and reduced excitability in the 4th month. The lack of reliable differences /88
between the test and control is evidently due to the wide range of individual values when excitability is determined with the use of brief stimuli.

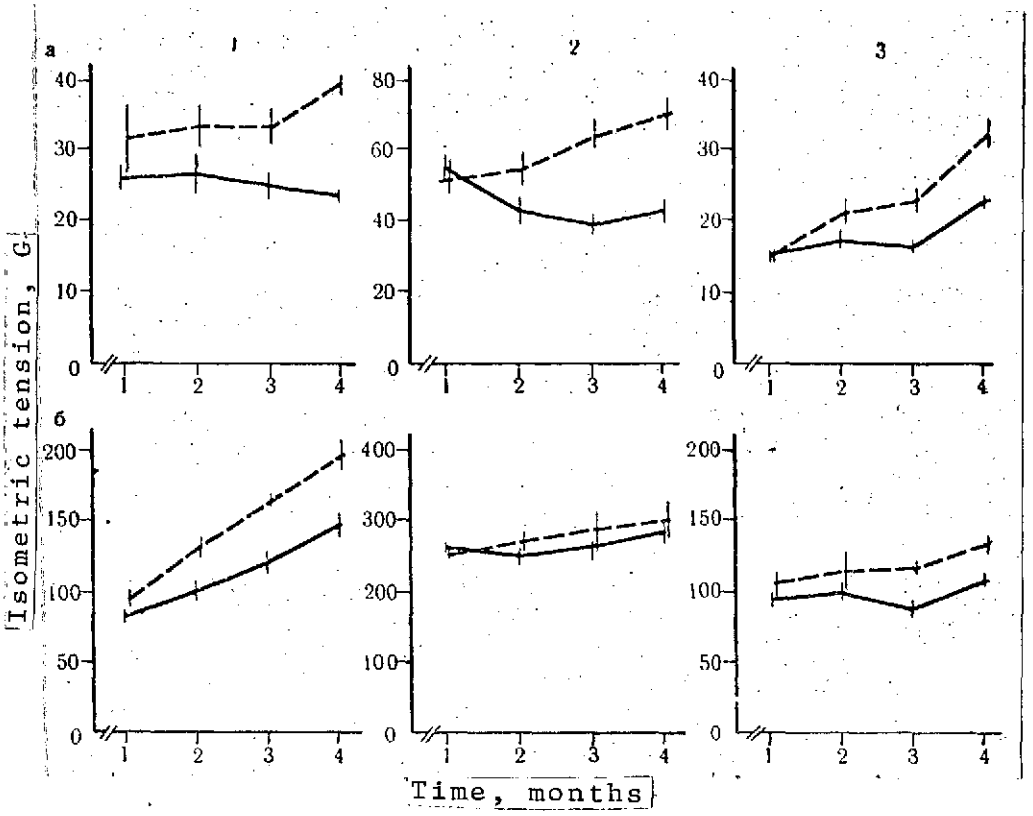

Figure 14. Changes in maximum amplitude of single (a) and tetanic (b) isometric responses in the extensor digitorum longus (1), soleus muscle (2) and plantar muscle (3) in test (solid curves) and in the control (dotted lines).

We determined temporal characteristics of isometric responses of muscles on the basis of such indices as frequency of superposition, frequency of fused tetanus and temporal parameters of single tetanic responses.

The frequency of superposition, i.e., minimum frequency of stimulation when response to the next stimulus begins before the previous one has ended, did not change throughout the entire period of hypokinesia in any of the muscles.

The frequency of fused tetanus, i.e., the frequency of stimulation when the plateau of isometric response loses its notched character, unlike the fre-

quency of superposition, during hypokinesia underwent significant changes. In the 3rd and 4th month of hypokinesia, in all muscles the frequency of fused tetanus was reliably lower than in the control.

Tension development time in a single response under the effect of hypokinesia was reliably increased in the 4th month in PM (11%). Half-life time of tension in a single response reliably increased in PM in the 3rd and 4th months (41 and 53% respectively). In the other cases temporal parameters of single responses remained unchanged.

The time for half development of tension with tetanic stimulation at a frequency of 100 pulses per second for 1 second increased under the effect of hypokinesia only in PM. The difference between test and control averages was reliable in the 3rd month (57±6.9 msec in the test and 37.8±3.9 msec in the control; $P < 0.02$) and unreliable in the 4th month of the experiment (58.4±4.7 msec in the test and 48.4±4.4 msec on the control; $P < 0.2$). In the other muscles this index did not change during the course of the experiment, with the exception of a reliable reduction in SM in the 1st month of hypokinesia to 22.0±1.6 msec in comparison with 27.0±1.7 msec in the control ($P < 0.05$). /89

Half-life time of tension after tetanic stimulation did not change significantly in SM and EDL during hypokinesis. In contrast, in PM, half relaxation time during hypokinesia increased (Table 14). A reliable increase in comparison with the control was noted in the 3rd month (31%) and in the 4th month (43%).

A study of the force characteristics of isometric responses showed that during the first month of the experiment, the amplitude of single responses by muscles of test animals did not differ from the control. In the 2nd month, the averages of this were lower in the test than in the control, but differences between each other were not statistically reliable. In the next periods of the experiment, the amplitudes of single responses were reliably lower in the test than in the control: in SM and PM — beginning in the

TABLE 14

HALF-LIFE TIME OF TENSION OF PLANTAR MUSCLE (IN MILLISECONDS) AFTER TETANIC STIMULATION AT A RATE OF 100 PULSES/SECOND FOR 1 SECOND

Month	Test	Control	P
1st	81.0±8.0	74.4±5.0	0.5
2nd	103.2±6.1	83.6±6.8	0.1
3rd	103.0±4.2	78.2±8.4	0.05
4th	109.2±7.2	76.0±2.0	0.001

3rd and in EDL - in the 4th month. Simple calculations showed that at these points, the ratios of amplitudes of single responses in the test to those in the control were lower than the ratios of corresponding weights. If this ratio for amplitudes of single responses in the 4th month of the experiment in EDL, SM and PM was 0.59, 0.62 and 0.7, the relative (test-control) weight of these muscles was 0.67, 0.70 and 0.78 g. This indicates that the reduced value of single isometric responses by muscles during hypokinesia was due not only to their lag in weight behind that of control muscles, but also to the absolute reduction of their force. Average amplitude of tetanic responses in EDL was lower than in the control beginning with the 2nd month and in PM from the 3rd month of hypokinesia.

In contrast, in SM throughout the entire experiment, no reliable changes were noted in the amplitude of tetanus in the test as compared with the control, although average amplitudes in the test, beginning with the 2nd month, were slightly lower than in the control.

Thus, hypokinesia is accomapnied by a complex set of changes in the function of skeletal muscles. Some of these are due, evidently, to the stress character of the experimental situation, especially at the beginning of the experiment on rats. /90

As the result of two-month hypokinesia, the rate of conduction of stimulation along fibers of the sciatic nerve decreased in rats.

The significant shifts in indices of functional activity of muscles in the second half of the experiment reflect, evidently, the effect of hypokinesia proper. And, actually, after the first month of hypokinesia, the motor activity of rats is reduced and continues to decrease at later points.

On the basis of these data it can be assumed that hypokinesia has a different effect on different neuro-muscular structures. In particular, if the frequency of fused tetanus, largely reflecting the functional lability of the neuro-muscular synapse, underwent the same type of changes in all muscles, the rate of weakening, reflecting the state of contractile elements, changed significantly only in slow plantar muscle. In addition, lack of significant changes in the time of a single contraction in the tested muscles agrees with earlier data on the absence of changes in ATPase activity of myosin in muscles of the leg during hypokinesia [17].

The basic tendencies of changes in the state of skeletal muscles during hypokinesia in our work are similar to those obtained by Z. I. Barbashova et al. [15] during 30-day hypokinesia. Their work showed a sharp reduction of maximum single and tetanic tension and an increase in the time of a single tension and weakening of the peroneal muscle during hypokinesia. The greater expression of changes in comparison with our experiment can be due to stricter immobilization of the animals, indicated by the sharp reduction in weight and high mortality of the animals noted in this work.

Finally, the data we obtained indicate that changes detected in man during hypokinesia and expressed as a reduction in the force of various muscle groups, disturbance of velocity properties and motor coordination [33, 90] can in part be due to disturbances in the control link of the neuro-muscular system.

In summarizing current experimental works by scientists in socialist countries on the problem of hypokinesia it must be noted that the participation of representatives of various specialties — morphologists, biochemists, physiologists and pathologists — has made it possible to expand research broadly and at the same time purposefully and has already produced a number of important data. These indicate that sharp limitation of motor activity causes a number of complex and interconnected processes, including those of a dystrophic and pathological character. Prevention of such changes during prolonged flights is the urgent task of space medicine.

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N75 14414

SPACE RADIOBIOLOGY /96

Justification of Permissible Doses of Radiation during Prolonged Space Flights

We are not able to evaluate possible radiation of spacecraft crews from positions of standardized professional practice. However, we must take into consideration the vast experience accumulated in many years of providing safe conditions for working with sources of ionizing radiation on Earth.

The radiation factor is a serious potential danger which must be taken into account in carrying out a prolonged space flight program. The danger of cosmic ray heavy ions for vital sections of the nervous system is considered specially (Table 15).

The radiation situation in space with regard to spatial and temporal distribution of dosage levels is extremely specific. An important aspect in /97
evaluating the danger is the modifying influence of other flight factors on the radiobiological effect.

Also of special significance in standardizing radiation for crews during prolonged space flight are such criteria as the working capacity of the cosmonaut during the entire flight and his mental activity. Special consideration must be given to the random character of radiation from solar flares. Thus, the probability of solar flares in a 15-day flight could be as follows:

Date of flare	Probability	Dose behind shield of 3.5 g·cm^{-2}
23/II/1956	0.01	34 rem
12/XI/1960	0.02	20 "
10/V /1959	0.02	14 "
3/X /1960	0.23	0.3 "

In connection with this, astronomers, physicists and radiobiologists are working out principles and programs for evaluating this danger.

TABLE 15

EVALUATION OF CELLULAR DEATH IN VARIOUS SECTIONS OF THE CENTRAL NERVOUS SYSTEM OF COSMONAUTS DURING THE FLIGHTS OF THE "APOLLO-12" AND TO MARS[1]

Section of the central nervous system	Diameter of cells	Diameter of nuclei	Proportion of dead cells per million cells	
	μμ		Flight of "Apollo-12"	Flight to Mars
Granular layer of the cerebellum	4	3.6	0.5	40-50
Retina	6x20	4	0.5-5.7	50-500
Cortex of the brain	19	4	2-14	16-120
Betz' cells	45	18	13-83	1050-6600
Anterior horn	70	25	26-200	2000-16000

[1]For integral flow are taken nuclei with Z > 10.

In justifying permissible levels of radiation during prolonged space flights, not only the so-called absolute risk due to radiation must be taken into consideration, but also the relative risk. The latter is determined by the ratio of the frequency of radiation injuries caused by radiation to analogous results due to other environmental factors. Solution of the problem of risk in evaluating the danger of possible radiation effects during prolonged space flights consists of finding the optimum ratio between the known radiation

danger and the advantages of the mastery of space for mankind. The level of inevitable effect must be so small that it is comparable to the corresponding danger of the space flight itself and the effect on man of harmful factors accompanying modern civilized society on Earth.

Depending on the situation developing during the space flight or a scheduled assignment, primary significance can be acquired by various criteria of risk. In connection with this, we must have available reliable material on the development of a whole complex of somatic effects during prolonged radiation of the body. Especially significant in this respect are the results of the "chronic experiment." /98

TABLE 16

CONDITIONS OF THE RADIATION OF DOGS IN THE "CHRONIC EXPERIMENT"

Characteristics of radiation effect	No. of group	Dose of chronic radiation per year	Dose and order of repeated acute radiations	Total doses for 5 years of radiation, rad				
		rad		1 year	2 years	3 years	4 years	5 years
Chronic radiation	1st	21	---	21	42	63	84	105
	2nd	63	---	63	125	190	250	315
	3rd	125	---	125	250	375	500	625
Combined radiation (chronic and acute)	4th	63	42+8+8=58	120	240	360	480	600
			42+42+42=126	190	380	560	750	940

The scientific program of this experiment specified study of the condition of so-called critical organs and systems of the body "responsible" for the course and outcome of radiation injury. 246 dogs were used which were divided into a number of groups depending on the directions of the study and radiation conditions. Chronic γ-radiation was conducted daily for 22 hours per day from Co^{60} sources in specially created units (some of the animals were radiated in

compartments and some on an open platform). Radiation of the major portion of the dogs was begun November 12, 1966. Corresponding data on groups of animals and test conditions are given in Table 16.

Studied during many years of radiating the animals were their general clinical condition, peripheral blood and marrow, the state of the cardio-vascular system, higher nervous activity, the state of the vestibular analysor, the organ of vision, spermatogenic function and the ability to reproduce, the state of natural and artificial immunity and a number of biological indices of the blood and tissues; cytological and histological tests were run as well as a number of other special studies.

This extensive and comprehensive work was made possible because of the wide participation in the experiment of a great many scientists from a number of institutes in the USSR and other socialist countries.

As the result of the work, extensive factual material was collected for 5 years, preliminary analysis of which indicates that chronic radiation of animals for 5 years with total dosage under 625 rad and combined radiation totaling under 940 rad did not cause any significant disturbances in the vital activity of dogs.

Several summaries have been published of biological studies of blood and tissues as well as of immunological reactions in dogs during the "chronic experiment" carried out by Hungarian, USSR and Czechoslovakian specialists.

At the present time, various ways and methods of protecting the crew from cosmic radiation are being developed. These are primarily methods of pharmcochemical and physical protection. The latter include protecting the spacecraft skin, on-board equipment and fuel supplies; the creation of a special anti-radiation shelter; development of magnetic and electrostatic protection. The /99
use of combined or local protective methods can be effective. However, solution of this problem is extremely complex, as space flight conditions impose very strict requirements on drugs, severely limit the weight and size of physical

protection, etc. At the same time, even a very slight reduction in the dose of possible radiation entails a very great increase in the weight of the craft. Thus, to reduce the total annual dose to 5 rem would require the creation of radiation shelters weighing about 100 g.

In connection with the above, it is, evidently, correct to speak of establishing a "dose of maximum permissible radiation" or a "dose of permissible risk," characterizing radiation in such dose which when effective throughout the flight does not cause changes in man and does not disturb working capacity. Of course, coefficients based on calculations must take into account the rate of development of recovery processes and constants must characterize permanent damage. Based on this, the following maximum permissible doses can be determined as preliminary values: 1 year of flight — 200 rem, 2 years of flight — 250 rem, 3 years of flight — 275 rem.

These summary levels of radiation are actually so-called effective doses for bone marrow. However, in prospective studies we must more accurately define the coefficients of recovery and permanent injury for combined radiation (chronic and fractionated) and evaluate the corresponding effects for a number of other critical organs (vestibular analysor, gonads, lens of the eye, skin, etc.). Such studies in the experimental justification of regulated doses of radiation during prolonged space flights are extremely time consuming, but they have been carried out on the basis of established traditions among scientists and radiobiologists of socialist countries working with the framework of "Interkosmos."

N75 14415

Study of a Number of Biochemical Indices
of the Blood and Tissue of Dogs after Prolonged γ-radiation.

The chronic irradiation of rats causes significant changes in the metabolism of lipids and glycides [48]. Until now, questions concerning changes in metabolic parameters in other kinds of laboratory animals during a prolonged

radiation effect have remained poorly investigated.

Radiation damage of hematogenic and lymphatic tissues, along with other changes, appears as an increase in the amount of soluble DNA (in a 0.14 M solution of NaCl) and a reduction in the amount of DNA contained in deoxyribonucleoprotein (DNP). The amount of DNA contained in DNP is reduced in relation to the dosage and the time elapsing after acute radiation. The maximum rate of reduction is observed on the first day. Chronic radiation causes milder changes. With chronic radiation of 1 and 10 R per day, the amount of DNA in the spleen, thymus and bone marrow of rats decreased on the 20th and 40th day, but recovery was noted on the 60th day [74].

In this experiment, the glucose content in the blood was studied and the lipid content in serum and tissues of dogs exposed to chronic radiation for 3 and 5 years [8]. In tissues of these animals, the concentration of soluble DNA and DNA contained in DNP was studied in the spleen, lymph node (deep cervical node) and bone marrow of thigh bones. /100

Adult dogs of all 6 "chronic radiation" groups were used in the experiment.[1]

To determine glucose in the blood and lipids in serum, blood was taken in vivo; lipids and DNA in tissues were determined after the animals were killed. Before they were killed the dogs were starved for at least 18 hours. Two dogs were killed from each test group and the control. In serum and the liver all basic lipid fractions were determined, in heart muscle and bone marrow — the concentration of triglycerides and phospholipids, in white fatty tissue — the concentration of unesterized fatty acids (UEFA), in the adrenals — cholesterol. The amount of soluble DNA (i.e. polydeoxyribonucleotides) and DNA contained in DNP was determined by the method based on the insolubility of DNP in 0.14 M solution of NaCl and the reaction with a diphenylamine reagent. Measurements were made on a spectrophotometer-SF4, wavelength 580 nm.

[1]For radiation conditions, see p. 143

In order to reveal sex-dependent differences in the biochemical indices of the animals, females were also studied.

The reliability of differences (according to criterion t) was determined by comparing data obtained in two control groups; in the other cases the arithmetical mean was calculated according to groups.

Basic results of studies of lipid exchange and glucose content are given in Tables 17-19.

TABLE 17

THE GLUCOSE CONTENT IN BLOOD AND LIPID CONTENT IN SERUM OF INTACT DOGS IN RELATION TO MAINTENANCE CONDITIONS AND SEX OF THE ANIMALS

Maintenance conditions	Sex	Blood		Serum		
		Glucose	Cholesterol	Phospholipids	Triglycerides	UEFA μeqv/l
		mg per 100 ml				
		++	+			++
Open area	M	67.8±2.0	123±8	278±17	57.4±5.5	976±60
Closed compartment	M	54.0±2.0	154±7	268±10	61.7±4.7	673±51
Open area	M	67.8±2.0	123±8	278±17	57.4±5.5	976±60
	F	64.4±5.0	139±7	320±20	57.4±4.9	952±78

Note. Statistical significance indicated by + ($P < 0.05$) and ++ ($P < 0.01$) with corresponding value.

In comparing data from two control groups, the following differences are revealed: increase in the glucose level of the blood, increase in the UEFA content and reduction in the cholesterol level in serum of the animals kept in an open area (see Table 17). It can be assumed that these changes have an adaptive character, i.e. they are the result of adaptation of the animals to changing environmental conditions. These data indicate the necessity of having two controls to evaluate long-term radiobiological experiments. Biochemi-

TABLE 18

CHANGE IN THE GLUCOSE CONTENT OF THE BLOOD AND THE LIPID CONTENT OF SERUM, ADRENALS AND WHITE FATTY TISSUE /101

Group	Length of radiation (in yrs)	Blood		Serum		Adrenals		White fatty tissue
		Glucose	Cholesterol	Phospholipids	Triglycerides	UEFA μeqv/l	Cholesterol mg/g	UEFA μeqv/l
		mg per 100 ml						
1st	3*	66.6	144	268	64.6	870	91.1	6.11
	5	72.1	147	267	60.8	1310	98.9	6.21
2nd	3	92.0	146	323	84.8	1071	74.7	4.47
	5	66.4	193	371	50.9	1195	41.7	4.11
3rd	3	70.8	124	289	77.9	1240	71.1	5.65
	5	83.5	143	298	79.8	1025	76.0	5.53
4th	3	76.8	163	315	52.8	805	90.3	5.77
	5	75.2	195	349	79.3	1021	56.1	4.17
5th	3	76.5	135	287	60.8	1320	61.1	4.75
	5	66.4	129	342	121.0	1405	95.1	5.33
Control	3	62.5	143	264	50.6	928	70.1	3.94
	5	67.5	117	296	56.6	1109	85.5	4.17

* One animal.

cal differences depending on sex, according to data of studies of control dogs from the open area, are slight (see Table 17).

Stable changes in the concentration of glucose in the blood and individual lipids in serum were not observed in any of the five groups of radiated animals. /102 The comparative equilibrium in the cholesterol content of the adrenals indicates the adaptation of the animals to chronic radiation (see Table 18). An increase in the content of triglycerides, phospholipids and cholesterol was observed in the liver, especially in the 3rd group and to a lesser degree in the 4th and 5th groups. A significant increase in the level of triglycerides with a simultaneous reduction in the level of phospholipids was noted in heart muscle, primarily in dogs of the 3rd group after 5-year radiation. Changes in bone marrow must be evaluated very carefully, as the content of fat in thigh bones increases greatly with age. Distinct individual differences were found in different dogs.

TABLE 19

CHANGE IN THE LIPID CONTENT OF THE LIVER, HEART MUSCLE AND BONE MARROW IN RELATION TO THE LENGTH OF RADIATION

Group	Length of radiation (in years)	Liver				Heart muscle		Bone marrow	
		Cholesterol	Phospholipids	Triglycerides	UEFA μeqv/l	Phospholipids	Triglycerides	Phospholipids	Triglycerides
		mg per 100 ml				mg per 100 ml			
1st	3*	6.19	28.2	6.10	2.04	24.0	5.90	25.1	451
	5	5.55	25.9	8.30	2.66	27.8	11.10	11.6	446
2nd	3	9.36	29.7	11.10	3.48	34.1	10.70	20.7	407
	5*	5.17	25.6	6.10	3.25	28.9	20.60	17.3	500
3rd	3	5.97	37.4	12.10	4.29	32.2	22.35	21.5	744
	5	9.98	41.1	18.70	3.44	23.8	33.25	10.7	574
4th	3	11.59	32.7	10.65	3.53	31.9	12.60	16.0	580
	5	7.88	31.3	17.35	3.43	27.1	13.25	14.6	472
5th	3*	10.10	32.2	19.60	3.58	31.7	17.70	10.1	494
	5*	9.40	25.9	10.90	3.06	31.9	17.40	9.0	774
Control	3*	5.67	25.1	12.30	4.26	36.0	10.70	12.8	405
	5*	7.31	33.5	10.70	3.56	35.2	9.90	11.0	384

* One animal.

Despite this we detected an increased content of triglycerides in the 3rd group after 3-year radiation and in the 5th group after 5-year radiation of the dogs (see Table 19).

The data indicate that under the influence of many years of chronic and combined (chronic + acute) γ-radiation, in the majority of test dogs, significant changes developed in the concentration of glucose in the blood and that of several lipids in serum and tissues. These changes were most pronounced in animals in the 3rd group, radiated chronically with the most powerful doses for 3 and 5 years. In animals of the 4th and 5th groups, exposed to a combined radiation effect, changes were slightly less than in the 3rd group. Deviations were insignificant in the 2nd group. In the 1st group of dogs with the lowest dose of chronic radiation, changes were very insignificant in comparison with the control and could be considered physiological variability. These deviations can be treated as the adaptive reaction of the body. An exception is

heart muscle, where the changes indicate organic damage to the tissue itself. The concentration of soluble DNA in all studied organs fluctuated within limits of the control value (Table 20). These results agree with data obtained in the chronic radiation of rats [74].

TABLE 20

CONCENTRATION OF SOLUBLE DNA (IN MILLIGRAMS PER GRAM DRY TISSUE) IN THE SPLEEN, LYMPH NODE AND BONE MARROW OF DOGS AFTER 3 AND 5-YEAR γ-RADIATION (AVERAGE DATA)

Group	Number of dogs radiated for		Spleen of dogs radiated for		Lymph node of dogs radiated for		Bone marrow of dogs radiated for	
	3 years	5 years	3 years	5 years	3 years	5 years	3 years	5 years
1st	1	2	0.175	0.119	0.076	0.103	0.01	0.073
2nd	2	1	0.211	0.168	0.021	--	0.122	0.102
3rd	2	2	0.183	0.184	0.056	0.012	0.024	0.071
4th	2	2	0.141	0.260	0.320	0.011	0.099	0.094
5th	1	1	0.191	0.177	0.007	0.270	0.320	0.011
Control	2	2	0.127	0.170	0.013	0.052	0.071	0.151

TABLE 21

CONCENTRATION OF DNP (IN MILLIGRAMS OF DNA CONTAINED IN DNP PER 1 GRAM DRY TISSUE) IN THE SPLEEN, LYMPH NODE AND BONE MARROW OF DOGS AFTER 3 AND 5-YEAR γ-RADIATION (AVERAGE DATA) /104

Group	Number of dogs radiated for		Spleen of dogs radiated for		Lymph node of dogs radiated for		Bone marrow of dogs radiated for	
	3 years	5 years	3 years	5 years	3 years	5 years	3 years	5 years
1st	1	2	6.14	5.01	9.80	9.97	0.33	1.19
2nd	2	1	3.96	3.48	8.11	--	1.55	0.57
3rd	2	2	3.17	3.35	7.76	6.79	0.54	0.59
4th	2	2	3.67	3.49	7.33	9.25	1.19	2.65
5th	1	1	4.86	4.37	7.45	5.97	0.85	2.04
Control	2	2	4.89	5.49	8.21	9.53	1.52	1.82

Changes in the concentration of DNP (based on the determination of DNA contained in DNP) in the spleen and bone marrow indicate their dependence on radiation conditions. The higher the daily dose of chronic radiation (2nd and 3rd groups), the greater the reduction in DNP concentration. With a radiation dose of 125 rad per year (3rd group) for 3 and 5 years, the amount of DNP in the spleen decreased approximately $^1/_3$ and in bone marrow almost $^2/_3$ of the control level (Table 21). The increased concentration of DNP in the spleen and the simultaneous reduction in bone marrow after 3-year radiation in the 1st group do not, in our opinion, have any significance, as these data were obtained from one animal. Combined (chronic + acute) radiation caused milder changes in the concentration of DNP (4th and 5th groups) than chronic radiation alone (2nd and 3rd groups). Changes in the concentration of DNP were slightly different in the lymph node than in the spleen and bone marrow. After 3-year radiation, the concentration of DNP was practically unchanged from the control level. Five-year radiation caused a reduction in DNP only after the use of the highest dose of chronic (3rd group) or combined (5th group) radiation. In comparing the amount of DNP in tested organs, it is evident that the greatest changes were found in bone marrow and to a slightly lesser degree in the spleen. The concentration of DNP did not undergo any marked changes in the lymph node.

The reduction in the concentration of DNP in tested organs reflects changes whose nature is difficult to explain on the basis of these data. Evidently, the reduction in the concentration of DNP (i.e. the basic amount of DNA in tissues) indicates a relative increase in the amount of non-nucleoprotein substances in organs and reflects, if only partially, changes in the relative number of cells with various nuclear-cytoplasmic ratios.

These data, therefore, indicate that the most significant changes in biochemical indices occurred in dogs which were exposed to chronic γ-radiation in doses of 125 rad per year.

N75 14416

A Study of Immunological Reactions in Dogs Exposed to Prolonged Chronic Radiation

/104

Predicting the condition of man during the effect of cosmic rays in a prolonged space flight, in particular that of mechanisms of anti-infection protection, is an extremely complex problem. More accurate definition of regulated doses for long space flights requires study of the sensitivity to the radiation factor of all basic systems of the body including the immunocompetent system.

Literature data on the effect on immunity of prolonged radiation in small doses (less than 5 R) is extremely sparse. N. P. Kiselev and P. A. Buzini [19] conducted chronic direct radiation of guinea pigs and random-bred mice in doses of 4.32 R/day. They showed that after 4-5 months of radiation, natural resistance is reduced. After 6 months the bactiricidal properties of the blood are reduced to complete disappearance. At the same time, a breakthrough of microbes into the blood stream was observed: with a total dose of 150 R organs and tissues in 14% of the animals were infected, with a dose of 950 R (after 5 months of radiation) bacteremia was noted in 64% of the animals and after a year in 90%.

For a number of years E. K. Dzhikidze [12] studied the effect of chronic radiation with small doses of ionizing radiation on infection and immunity in primates, as well as in laboratory animals (guinea pigs, rabbits, mice). The author showed that with fractional daily radiation of the animals (with the exception of week-ends) in doses of 1 and 4 R per day, signs of chronic radiation sickness gradually appeared. Dzhikidze noted signs of increased proliferative activity of bone marrow cells in monkeys after several months of radiation. As dosage accumulated, activation gradually changed to depression of proliferative processes in lymphopoietic tissue and its depletion of lymphocytes. Prolonged radiation of monkeys with x-rays in doses of 1 and 4 R caused various infection complications which became the cause of death of the animals even in small total doses when signs of radiation sickness were absent

or very weak. Bacteremia developed in some cases. With a total dose of 500-600 R (chronic radiation of monkeys for 5-6 months at a dose rate of 4 R/day or 1½-2 years of radiation at a dose of 1 R per day), damage to antibody formation was observed.

These few experimental observations of a number of authors show that prolonged chronic radiation with small doses of ionizing radiation (continuous or fractional) in doses of 1-5 R per day leads to a steady disturbance of immunological mechanisms; with a rather prolonged effect this causes death of the animals from endogenic and exogenic infections.

In our research we studied several indices of immunological reactivity of dogs, which for several years had been exposed to continuous γ-radiation from Co^{60} sources.

In this experiment we set ourselves the task of studying the formation of several reactions of specific immunity — the dynamics of the formation of humoral antibodies, the proliferation and differentiation of antibody-synthesizing cells, as well as several other indices of processes underlying the formation of active specific immunity. /105

The studies were conducted on 95 dogs (males and females) taken from all 5 test groups.

Immunization. 23 dogs were given single subcutaneous injections of 0.5 ml of a 50% suspension of sheep erythrocytes. Blood for analysis was taken before immunization and then on the 5th, 10th, 15th, 21st and 29th days. The other 24 dogs were immunized with native diphtheria anatoxin subcutaneously into the heel of the left hind limb (160 Lf) and simultaneously intramuscularly (40 Lf). Immunization was repeated in 21 days. Blood for analysis was taken before the administration of the antigen, on the 7th day after the first immunization, before the second injection of antigen and upon sacrifice. The animals were killed on the 4th day after the second stimulation; regional

lymph nodes and the spleen were removed immediately after death.

Lymph nodes were fixed and imbedded in paraffin according to the St. Mary method. A suspension of cells was prepared from pieces of spleen; 32 dogs were injected with a lyophilized preparation of phosphorylase-B extracted from the muscle tissue of roosters according to the method of Fisher and Krebs. In the repeated stimulation (after 5 months) the antigen was injected without an adjuvant.

Serological methods. The titer of total hemagglutinins is determined by the direct hemagglutination method. Antibodies against diphtheria anatoxin were determined by the passive hemagglutination method according to Boyden. For each test there was a parallel control of specific inhibition of hemagglutination.

Antiphosphorylase activity of serum was determined according to the Iokay and Kartzag method.

Incubation with a labelled precurser (uridin-C^{14}). The isotope label method in vitro was used to compare the rate of RNA synthesis in cells of lymphoid organs in test and control animals. Autoradiographic analysis of the rate of DNA synthesis and immunological analysis were made.

PGA-blast transformation of lymphocytes in peripheral blood was evaluated periodically in 18 dogs (12 radiated and 6 controls) for 6-7 months.

Results were analyzed statistically by the t-criterion method of Student as well as by the confidence interval method.

The formation of humoral antibodies was studied in three series of tests.

The first response after immunization with sheep erythrocytes was studied in dogs which were radiated for 2½ years with doses of 75 rem per year as well

as 75 rem per year with three additional acute radiations (50 rem each; a total of 225 rem per year). The titer of total, as well as cysteine-resistant, hemagglutinins did not differ at any point of radiation in the control and test.

In determining the titer of antibodies against diphtheria anatoxin there were also no significant differences in the serum of 24 test and control dogs. Some tendency toward increased titer was noted with doses of chronic radiation of 150 rem per year; these changes were more pronounced in dogs radiated for 2 years than in those radiated for 4 years.

Antiphosphorylase activity of serum was determined in 32 dogs of which 10 were a control group; the two remaining groups (11 dogs in each) were radiated

TABLE 22

/106

DETERMINATION OF THE LEVEL OF ANTIBODIES AGAINST PHOSPHORYLASE IN SERUM OF DOGS AFTER CHRONIC RADIATION (STATISTICAL ANALYSIS BY STUDENT'S t-CRITERION)

Time after second immunization (in days)	No. of group of dogs	Dose of radiation, year, rem	Total dose of radiation for 3 years on the day of analysis	Average level of antibodies (units of antiphosphorylase activity)	M ± m	t		P
11	1st	--	--	908	2.90±0.10	--		
	2nd	75	225±2.2*	552	2.66±0.12	1.50		
	3rd	75	225	691	2.82±0.10	0.57	>	0.05
	4th	150	450±4.5*	830	2.89±0.01	0.03		
	5th	150	450	301	2.40±0.13	3.13	<	0.05
33	1st	--	--	459	2.66±0.05	--		
	2nd	75	225±6.6*	280	2.40±0.09	2.60		
	3rd	75	225	410	2.61±0.01	0.98	>	0.05
	4th	150	450±13.5*	501	2.58±0.05	0.40		
	5th	150	450	174	2.15±0.14	3.06	<	0.05

*Conventional symbol for dose of radiation for group of dogs which continued to receive radiation during the examination period.

with doses of 75 or 150 rem per year. In addition, within each test group, 6 dogs continued to receive radiation after immunization, while radiation of the other 5 was stopped before start of the immunological study. Results of the determination of the level of antibodies against phosphorylase-B are given in Table 22. A statistically reliable reduction of antiphosphorylase activity was found only in the group of dogs radiated in doses of 150 rem per year where radiation was stopped before immunization. On the 11th day, the average level of antibodies in this group of dogs was 3 times less than in control animals. At later points (a month after the second immunization) a slight decrease appeared in the inhibition of antibody formation in this group of dogs; however, in comparison with the control serums, the average antiphosphorylase activity as before remained significantly reduced (according to average figures — 2.6 times); the difference is statistically reliable at $P < 0.05$. In the other groups of radiated dogs, the level of antibodies in serum did not significantly differ from the control (Fig. 15).

Immunomorphological study of regional lymph nodes showed the following. In all tested dogs (22 animals) on the 4th day after the second immunization in regional lymph nodes staining by the indirect Kuntz method revealed a significant number of antibody-synthesizing cells. The main mass of cells was in medullary cords; in the cortical zone their number was much less (tens of cells per slice). In analyzing the number of antibody-synthesizing cells it was found that in regional nodes of control animals, several hundred antibody synthesizing cells appeared per slice. In test dogs under radiation, the number of cells containing diphtheria antibodies was sharply increased.

In dogs which were exposed to combined radiation (total doses of 145 and 225 rem per year) for 2 years, as well as in dogs chronically radiated for 4 years, the number of cells containing specific antibodies exceeded the control level by an order of magnitude. /107

In the group of dogs which were radiated 2 years in doses of 75 and 150 rem per year, an even greater increase was observed in the number of antibody-containing cells (Fig. 16).

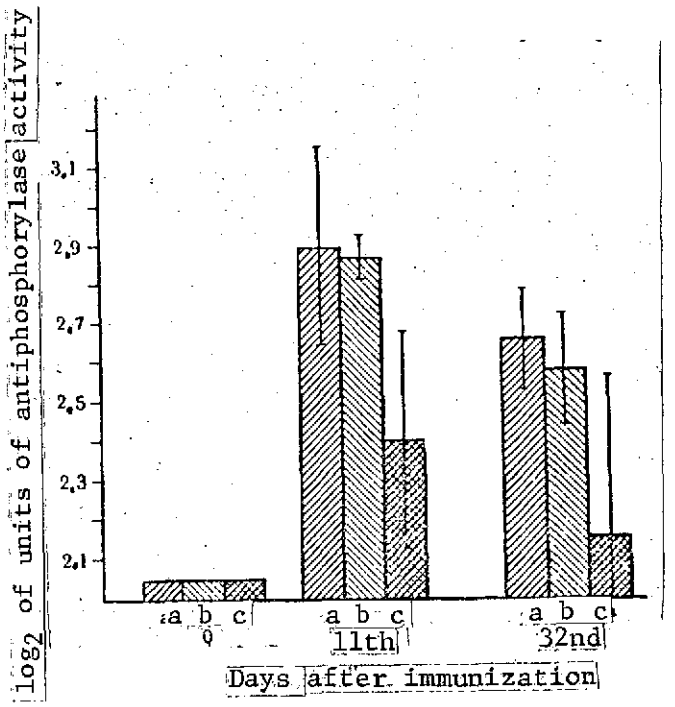

Figure 15. Change in antiphosphorylase activity in the blood serum of dogs after prolonged chronic gamma-radiation in doses of 150 rem per year.

a — control (no radiation); b — radiation during examination; c — radiation stopped before second immunization.

In comparing ratios of the number of cells synthesizing specific antibodies to cells containing nonspecific immunoglobulins, it was found that for every antibody-forming cell in control animals there were up to 20-50 cells synthesizing nonspecific immunoglobulins (1:20-1:50). In dogs of the 3rd, 4th and 5th groups, this ratio increased to 1:5-1:10, and in animals which for 2 years were exposed to chronic radiation alone in doses of 75-150 rem per year, this ratio was only 1:1.5-1:3.

Analysis of formulas of different types of antibody-synthesizing cells showed no statistically significant differences in any group of animals.

In studying the morphological picture of regional lymph nodes stained with methyl green-pyronine, noted in radiated dogs were a very slight contraction of the cortical zone and in increase in dimensions of pulpy cords, a significant increase in the number of pyroninophilic cells located primarily in the medullary cords in large groups — 50-100 cells each. Breeding centers of secondary nodules were weakly active in both control and test animals.

The rate of RNA synthesis in cells of the spleen was determined in 22 dogs killed on the 4th day after the second immunization with diphtheria anatoxin. Test animals were from four different groups (radiation doses — 75, 150, 145 and 225 rem per year).

/108

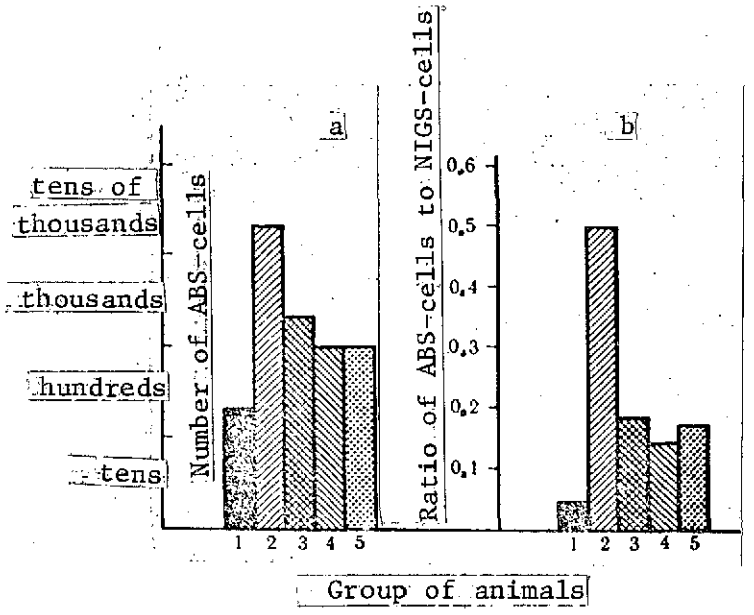

Figure 16. Quantitative characteristics of populations of antibody synthesizing (ABS) cells and cells synthesizing nonspecific immunoglobulins (NIGS) in regional lymph node of dogs after chronic radiation

a — number of ABS-cells per slice with a correction for the size of the lymph node; b — ratio of the number of ABS-cells to the number of NIGS-cells; 1 — control (no radiation); 2, 4 — chronic radiation of 75 and 150 rem/year for 2 and 4 years respectively; 3, 5 — combined radiation (total 50 rem) for 2 and 4 years respectively.

Calculation of the radioactivity of cellular suspensions (20×10^6 cells per test), incubated with uridin-C^{14} showed that the intensity of RNA synthesis in spleen cells of radiated and non-radiated animals, both immunized and intact, did not differ.

In calculating the content of cells synthesizing DNA, it was established that the label index in radiated and non-radiated dogs is extremely stable (1%). PGA-blast transformation of lymphocytes in peripheral blood was studied

in 18 dogs of which 12 animals were exposed to chronic radiation for 3 years in doses of 75 rem per year and 225 rem per year (6 dogs in each group); the other 6 dogs served as a control.

TABLE 23

DETERMINATION OF THE PERCENT OF BLASTS IN A 24-HOUR CULTURE OF NUCLEAR BLOOD CELLS OF DOGS, CONTROLS AND THOSE EXPOSED TO PROLONGED CHRONIC RADIATION (3 YRS)

Incubation of cells	Radiation of dogs	Percent of blasts		
		M ± m	t	P
With PGA	No radiation	4.15±0.74	-	-
	775 rem per year	7.30±2.02	1.46	>0.05
	225 rem per year	17.4±2.99	4.30	<0.01
Without PGA	No radiation	0.70±0.22	-	-
	75 rem per year	0.85±0.35	0.40	>0.05
	225 rem per year	2.60±0.58	3.10	<0.05

In some animals, analysis was conducted 2-3 times in 6-7 months of observation. Fluctuations in the percent of transformed cells in the same animals were insignificant in the majority of cases. /109

Results of calculating the percent of blasts in cultures with the addition of PGA are given in Table 23. As it shows, activation of the blast transformation reaction was found in dogs exposed to prolonged chronic radiation. With radiation doses of 225 rem per year (75 rem per year with three additional acute radiations of 50 rem each), the percent of blasts in a 24-hour culture was 4.2 times greater than in the control group (difference statistically reliable). In calculating the percent of blasts in parallel cultures (without the addition of PGA) a significant "spontaneous" transformation was found in the group of dogs receiving 225 rem per year.

The following facts which we obtained must, therefore, be compared:

1) lack of changes in radiated dogs of the first humoral response to erythrocytes and the second response to weak antigen (phosphorylase-B);

2) increase in the number of antibody-forming cells and total number of pyroninophilic cells in regional lymph nodes of radiated animals with the second injection of diphtheria anatoxin;

3) a distinct increase in the PGA-blast transformation reaction of lymphocytes in peripheral blood in vitro;

4) lack of changes in the rate of RNA synthesis in spleen cells of radiated and non-radiated animals which either received antigen or did not;

5) lack of changes in the content of cells synthesizing DNA in the spleen of radiated and non-radiated animals;

6) stable inhibition of the secondary immunological response to phosphorylase antigen in animals after the end of prolonged radiation.

The stability of indices of RNA synthesis and the number of cells synthesizing DNA in animals receiving and not receiving antigen leads to the conclusion that in this test situation (dose and place of administration of anatoxin) there was minimal participation of the spleen of dogs in the reaction to secondary stimulation. At the same time it is evident that by itself, prolonged radiation did not cause a reliable change in the synthesis of nucleic acids in spleen cells.

Evidently, we must consider absence of changes in humoral antibodies during the primary response to strong antigen (heterological erythrocytes) and the secondary response to weak antigen (phosphorylase) as symptoms of the same order.

With the use of a strong antigen (native diphtheria anatoxin), a distinct increase is immunomorphologically revealed in the number of cells synthesizing specific antibodies, as well as in all pyroninophilic cells in the regional lymph node.

Especially distinct is the increase in the number of cells synthesizing specific antibodies (Kuntz method) as well as all pyroninophilic cells in the lymph node found in dogs after 2 years of radiation in the smallest doses (75 and 150 rem per year without additional acute radiations). With increase in the length of the effect or with more intensive radiation (additional acute radiation against a background of a continuous effect) stimulation was less, /110 although it reliably exceeded the control level. We tried to study the mechanism of this stimulation of antibody formation.

In analyzing ratios of various types of antibody-synthesizing cells by the Kuntz method, reflecting the course of differentiation of these cells, it was found that formulas of antibody-forming cells did not statistically differ in animals of all test and control groups. This indicates the uniform differentiation of antibody-forming cells in animals. On the basis of these data, it can be assumed that stimulation of antibody genesis in chronically-radiated animals is basically due to the increased number of precursors of antibody-forming cells appearing in differentiation after the second antigenic stimulus.

There is significant interest in the fact that lymphocytes in a blood culture of radiated dogs in vitro with the addition of PGA gave a more pronounced response than the control cultures.

As is known, during the first day under the effect of PGA in vitro, the synthesis of RNA and protein is activated, but synthesis of DNA and proliferation have not yet begun. It is, therefore, obvious that in the test, a significantly larger part of peripheral lymphocytes reacted to the blast-transforming component of PGA than normally. The increased content of PGA-reactive cells in the population correlates to the increased number of cells in the lymph node beginning to differentiate under the effect of the antigen. These facts are of interest in connection with the simple correlation between the state of immunoreactivity due to the presence in the blood stream of thymus-instructed cells and results of the PGA-test [55].

This ratio between elevated reactivity of cells to the antigen and PGA is of interest in interpreting the biological significance of the PGA-test, in particular, changes in PGA-blast transformation during the effect on the body of other extreme factors [24].

In our tests we also found the symptom of "spontaneous" blast transformation of lymphocytes, expressed by the cultivation of cells in peripheral blood without the addition of PGA. This phenomenon also indicates the truth of the above stated theories that chronic radiation in small doses of ionizing radiation alters the properties of the cell population of lymphoid tissue which, in particular, is expressed by the reaction of a larger number of cells to antigen and to PGA. In connection with this, of special interest are observations of animals to study the second immunological response soon after termination of prolonged 3-year radiation. In animals of this group a distinct, statistically reliable increase was found in response to the injection of the antigen. At first glance this paradoxical fact will force us to assume that inhibition of the reaction of lymphoid cells to antigen in comparison with animals whose radiation was not stopped, as well as in comparison with the control (not exposed to radiation), bears largely an active character.

At present it is difficult to make any simple judgment in relation to the mechanism of this "active inhibition." At the same time, working hypotheses can be advanced which we are unable to prove.

It is very probable that chemical products formed in the body (in lymphoid cells) with the chronic effect of extremely small doses of ionizing radiation /111
(0.2-0.4 rem/day) are somehow responsible for activation of the specific response of lymphoid cells with the second administration of a strong antigen and further increase in blast transformation of lymphoid cells in vitro. The mere fact of the absence of an increase in primary response to a strong antigen and a secondary response to a weak antigen is not sufficient basis for assuming that some mechanism appears (or occurs) in the body, compensating for the unfavorable effect of chemical products of ionizing radiation (radicals, ions)

in the function of lymphoid cells. However, such an assumption seems natural in evaluating the results of a group of tests on animals in which radiation was terminated shortly before the study and where inhibition of the immune response was observed.

It seems important to us to undertake future searches to find this assumed mechanism, as its role in the compensation of aftereffects of chronic radiation of the body with small doses of ionizing radiation can go far beyond the limits of physiology of lymphoid tissue and the immune response.

These observations must also be compared with a group of still poorly investigated facts united under the common name "readaptation of the body after the effect of extreme factors."

N75 14417

Radiobiological Effects of Heavy Ions and Protons

The study of radiobiological effects of radiations with high linear energy transfer (LET) is at present an extremely urgent task. In this sense, there is significant interest in studying the radiation reaction of biological objects at various levels of biological organization to radiation with large LET.

In our experiments we studied the radiobiological effects of heavy ions and protons on cells of mammals, bacteria, viruses and DNA of bacteria. Material existing in the literature regarding radiobiological effects of densely ionizing radiations shows that the character and course of damages significantly differ from those from "standard" radiations. These differences primarily concern an increase in the relative biological effectiveness of densely-ionizing radiations and lack of or weak expression of post-radiation recovery processes.

In the present chapter are given data on the radiosensitivity of liver cells of rats, epithelium cells of the cornea of mice, bacteria E. coli B,

phages C_D and λ_{11}, as well as transformation DNA under the effect of heavy ions and protons. In order to study the mechanisms of the biological effect of ionizing radiations, we also studied the rate of nucleic acid synthesis in cells of a regenerating rat liver, radiated in a state of rest, with simultaneous calculation of changes in the state of the cellular population.

In experiments with cells of the liver and epithelium of the cornea of mice, we used accelerated carbon ions and boron ions at a dose rate of 10 rad/sec. The energy of particles entering a biological object was 3.3 and 5.7 Mev/nucleon. The physical characteristics of radiations used in experiments with micro-objects are given in Table 24. /112

TABLE 24

PHYSICAL CHARACTERISTICS OF KINDS OF RADIATION USED

Kind of radiation	Energy of radiation, Mev/nucleon	LET Mev·cm^2/g	Dose rate rad/sec	Source
H^1	645	2.4	30	Synchrocyclotron
B^{11}	7.3	14,400	500	
B^{11}	1.5	3,900	500	
C^{12}	5.8	2,300	500	
C^{12}	3.5	3,500	500	UMZI-300
Ne^{20}	8.4	5,000	500	
Ne^{22}	3.2	9,000	500	
Ar^{40}	1.5	30,000	500	
He^4	1.0	1,150	10.4	Am^{241}
γ-rays	--	3.0	15.5	Co^{60}, PX-γ-30

In studying the cytomorphological characteristics of damage to liver cells, white random bred rats of both sexes weighing 160-180 g were used. Under ether narcosis the liver was brought to the anterior abdominal wall, the animal was immobilized and the left lobe of the liver radiated with carbon ions in a dose of 300 rad or x-rays in doses of 1000 and 3000 rad. After 15 and 30 days the animals were killed. In histological specimens, the number of cells with preserved nuclei in 10 fields of view of each specimen were counted,

the diameter of nuclei measured and morphological changes described.

The biological effect of heavy ions on cells of the epithelium of the cornea was studied in random bred mice weighing 16-18 g. The animals were exposed to radiation with carbon and boron ions, as well as x-rays in a dose range from 25 to 500 rad. In 24 and 72 hours and 1, 2 and 6 months after radiation the animals were killed. From the enucleated eyeball, total specimens of the cornea were prepared which were stained according to the Karachi method.

As the object of study in experiments with labelled precursors of nucleic acid were used white male rats weighing 190±20 g. The animals were exposed to local radiation with 250 kV x-rays in the area of the liver in doses of 100, 300, 500, 700 and 900 rad. The rest of the body was protected by lead shields. Partial hepatectomy was performed 24 hours after radiation. Six hours after the operation, as a labelled precursor of nucleic acids, the animals were injected with P^{32} and C^{14}-orotic acid. The specific activity of DNA and RNA was recorded 30 days after the hepatectomy; the number of cells taking part in mitosis, the phase distribution of mitosis and the presence of structural breaks in chromosomes were determined.

The methods used in experiments with bacteria and bacteriophages have been described previously [28]. The number of vir-mutants was determined by seeding radiated phages λ_{11} on a bed of cells of E. coli K = 12λ. Control experiments showed no effect of tracer escape for all kinds of radiations. Mutants of phage C_D were identified visually by the morphology of negative colonies. In doubtful cases, phages were subjected to a repeated inoculation. /113

The donor of transforming DNA was a culture of B. subtilis ind^- $hist^-$. Transformation DNA was obtained from the biological mass of a 7-hour culture of the donor strain. Cells were subjected to lysing with lysozyme on the basis of 200 μg per 1 ml and were deproteinized with the subsequent double

effect of 6 and 4% PASA, an equal volume of phenol and chloroform-iso-amyl alcohol. RNA was removed with the help of RNA-ase (50 µg.ml). Sedimentation was conducted with 96% ethanol. After exposure, 0.1 ml DNA was added to 0.9 ml of the 18-hour culture which was competent and cultivated for 48 hours in minimal agar containing tryptophan.

The damaging effect of accelerated carbon ions on liver tissue of rats appeared in the development of destructive-degenerative disturbances, a change in the number of cells and the diameter of their radia. The depth and severity of developing changes depended on the dose and observation time and differed with significant individual fluctuations. In some animals in the acute period of radiation injury, liver tissue underwent necrosis with subsequent replacement by connective tissue and in other cases showed only dystrophic changes. In such cases, the cytoplasm of hepatocytes underwent unique changes. Its substance appeared as individual pieces, threads or coarse lumps, among which the nucleus was freely located. As heavy ion radiation was not deep, more significant dystrophic changes were suffered by the surface layers of renal cells, while with x-rays dystrophic disturbances bore a diffuse character. Often in the liver of animals radiated with carbon ions a distinct boundary could be seen between damaged and healthy tissue in the form of a layer of deep dystrophic changes. Calculation of the number of hepatocytes in radiated and healthy tissue and measurements of the diameter of their nuclei are given in Table 25. As can be seen from this table, radiation of the liver with heavy ions in a dose of 300 rad led to the death of actually half of the hepatocytes by the 30th day after radiation.

Radiation of the liver with x-rays in a dose of 1000 rad did not cause a reliable reduction in the number of hepatocytes by this point. Only the effect of x-rays in doses of 3000 rad caused a reduction in the number of cells, but it was less pronounced than in the case of carbon ion radiation. As by the /114
15th day after the effect the number of hepatocytes remained close to the control level, it can be assumed that the first 30 days after radiation is the optimum period for radiation damage. Along with the decrease in the total number

TABLE 25

CHANGE IN THE NUMBER OF CELLS AND THE DIAMETER OF THEIR NUCLEI IN THE LIVER OF RATS IN RADIATION WITH CARBON IONS AND X-RAYS

Kind of radiation	Dose, rad	Times of study (in days)			
		15		30	
		Number of cells	Diameter of nuclei	Number of cells	Diameter of nuclei
Carbon ions	300	94±8	6.98±0.09	67±4	7.21±0.09
X-rays	1000	90±6	8.20±0.19	113±14	6.92±0.14
	3000	121±7	7.62±0.09	86±11	8.41±0.16
Control	--	118±7	8.38±0.19	116±24	7.93±0.46

of cells there was a reduction in the diameter of nuclei of liver cells. It was more pronounced by the 15th day after radiation and was also more severe after the effect of heavy ions. Whether these changes in the diameter of hepatocyte nuclei were forerunners of their death is at present still unclear; however, it can be assumed that these cells did receive lethal injuries. In this case it can, evidently, be a matter of the so-called interphase death of hepatocytes due to organic and functional disturbances developing in radiated tissue. It is known that at later and remote points after radiation, destructive-degenerative disturbances develop in the liver of the animals; the degree of such damage depends on the radiation dose and observation times. Some cells undergo irreversible destructive changes and gradually die, which we also had the opportunity to observed in 30 days after radiation.

Although renal tissue is highly radioresistant, serious disturbances develop in it during local radiation with carbon ions in doses of 300 rad. They indicate that heavy ions are 3-10 times more effective than x-rays, but realization of a biological effect requires a comparatively long period of time (15-30 days).

TABLE 26

CHANGE IN THE FREQUENCY OF ABERRANT MITOSES IN CELLS OF CORNEAL EPITHELIUM OF MICE AFTER RADIATION

Kind of radiation	Dose, rad	Aberrant mitoses				
		24 hrs	72 hrs	1 month	2 months	6 months
X-rays	50	14.9±0.5	7.8±0.4	1.9±0.1	1.3±0.3	1.0±0.2
	100	22.5±0.5	15.6±0.8	3.1±0.4	2.1±0.1	1.3±0.4
	250	41.5±0.1	31.2±0.3	7.2±1.2	4.4±0.1	2.4±0.3
	500	59.3±1.9	61.7±0.6	11.7±0.9	8.1±0.7	4.6±0.6
	-	0.7±0.1	1.0±0.1	1.1±0.1	1.0±0.3	0.9±0.1
Carbon ions	25	20.5±0.6	29.6±0.7	23.4±0.4	22.3±1.3	14.6±1.1
	50	35.5±0.8	46.7±0.5	40.1±1.2	-	-
	100	52.1±1.0	60.3±0.8	-	34.8±1.6	22.8±1.9
	250	72.6±0.9	73.9±1.0	-	-	-
	500	80.3±1.1	-	-	-	-
Control	-	0.8±0.1	0.8±0.1	0.9±0.1	0.7±0.1	0.6±0.1
Boron ions	25	23.3±0.8	-	-	20.2±0.7	-
	50	39.2±1.8	-	-	30.8±0.5	-
	100	56.1±1.8	-	-	35.5±0.6	-
	250	68.7±1.8	-	-	59.8±1.0	-
Control	-	0.9±0.1	-	-	0.6±0.03	-

Results of a study of cells of the corneal epithelium are given in Table 26. These materials show that the content of aberrant cells after radiation with carbon ions remains high for a long time. This phenomenon indicates extremely low intensity of recovery from radiation injuries caused by heavy ions and significantly increases RBE coefficients if the latter are based on a comparison of remote effects. /115

As seen from Table 26, the content of aberrant mitoses 2 months after radiation with carbon ions in doses of 25 rad exceeds their level during the effect of x-rays in doses of 500 rad. Thus, RBE coefficients of carbon ions according to the criterion of the content of aberrant mitoses 2 months after radiation were not less than 20. This relates equally to accelerated boron ions. By the 6-month point of observation, the content of aberrant cells was insignificantly reduced.

Analysis of materials obtained in studying the mitotic activity of radiated cells in a regenerating rat liver and their phase distribution in mitosis showed that x-rays in a dose range from 100 to 900 R delay the start of the first post-radiation mitosis. With increase of radiation dosage from 100 to 900 R the mitotic index was reduced almost in half. Analysis of the distribution of fissionable cells by phases of mitosis revealed a significant predominance of metaphase.

This study of DNA and RNA synthesis in quiescent cells of an intact rat liver using P^{32} and C^{14}-orotic acid revealed the metabolic stability of nucleic acids. Sharp intensification of the biosynthesis of DNA in the regenerating liver of non-radiated animals was determined 24 hours after hepatectomy when the specific radioactivity of DNA practically doubled.

Results of the study showed that radiation of a section of a rat liver with various doses significantly repressed the rate of inclusion of P^{32} in DNA. Reduction of the intensity of DNA synthesis more than half in comparison with the intact operated control was already noted with a dose of 100 rad and remained at approximately this level during radiation in doses to 900 R.

With regard to the intensity of RNA biosynthesis, it was reduced by radiation in doses of 100 rad with subsequent increase with doses of 300 R and return to values typical of the intact operated control with doses of 900 R.

The liver of mammals, as tissue with extremely low mitotic activity, is considered as radioresistant tissue. However, our data show that the radioresistance of liver tissue is extremely relative. In a locally-radiated normal liver there are a number of serious changes connected primarily with damage to the cell nucleus. With the effect on liver cells of radiations with high density ionization, destructive-degenerative processes are, evidently, even more pronounced.

In experiments with bacteria, phages and transforming DNA, radiobiological effects of corpuscular rays, having various charges and energies, were studied.

Table 27 gives data on the inactivation of the transforming activity of DNA, radiated by protons in vitro and in vivo. Analysis of data shows that

TABLE 27

/116

RESIDUAL TRANSFORMATION ACTIVITY OF DNA RADIATED WITH VARIOUS DOSES OF PROTONS IN VITRO AND IN VIVO

Dose, krad	Residual transformation activity, %							
	radiation				radiation			
	Without cystamine	P	With cystamine	P	Without cystamine	P	With cystamine	P
5	87	<0.05	87	>0.05	--	--	--	<0.05
10	73	<0.05	93	>0.05	93	<0.05	99	<0.05
20	53	<0.05	87	<0.05	75	<0.05	91	<0.05
50	23	<0.05	64	<0.05	40	<0.05	85	<0.05
100	4.9	<0.05	37	<0.05	22	<0.05	72	<0.05
112	3.9	<0.05	31	<0.05	15	<0.05	66	<0.05

as the radiation dose is increased, the ability of DNA to change the sign of tryptophan-independence is reduced. In graphic form this dependence bears an exponential character. With a dose of 5 krad, the transforming activity of DNA is 87% of control levels, while with a dose of 112 krad, this is reduced to 4%.

As can be seen from Table 27, cystamine in our model has a distinct radioprotective influence in relation to the inactivating effect of protons. The reduced dosage factor (RDF) here is significant — 3.25.

Analysis of materials obtained in radiating DNA in vivo also reveals dose dependence, graphically described by an exponent, as in the case of radiating DNA in solution. With a dose of 10 krad, residual transforming ac-

tivity is 93% and with a dose of 112 krad — 15%. Our data verifies the theory that in dissolved form, DNA is more sensitive to the effect of radiation. Radiation of DNA in vitro in the presence of cystamine also gives a radioprotective effect with RFD of 3.5.

In experiments with bacteriophages, both dry and bouillon specimens were exposed to radiation. Results of these experiments are given in Fig. 17. In the entire interval of studied doses up to survival of 10^{-5} for both dry and bouillon specimens, dose dependences of inactivation are single-stroke. For dry specimens, D_{37} of γ-rays is 110 krad and for protons 120 krad (Fig. 17, curves 3 and 4), i.e., in this case, protons have a lesser biological effect than γ-rays. For bouillon cultures, D_{37} for γ-rays and protons was 26 and 32 krad respectively.

The kinetics of mutagenesis of phage C_D are given in Fig. 17, describing the linear dependence of mutant production on radiation dose for both dry and bouillon specimens. It is important to point out that the frequency of mutations is not affected by the kind of radiation but the condition of specimens significantly affects the degree of mutagenesis for the same doses.

Fig. 17 also gives data on inactivation and mutagenesis of phage λ in the form of dry specimens during the effect of γ-rays and accelerated carbon and neon ions. This illustration gives dose dependences of inactivation and mutant production during radiation of bouillon specimens with γ-rays and protons. These dependences also bear single-stroke characters for the studied range of doses. / 117

The points on curve 4 correspond to inactivation of phage λ_{vir} with C^{12} ions according to titration in strain E coli K 12 (λ). We isolated phage (λ) from phage λ_{11} of C and, thus, it has the same molecular weight; therefore, coincidence of the curves of inactivation of phages λ_{11} and λ_{vir} can be considered proof of the lack of effect of tracer vir escape for this kind of radiation. The linear dependence of the rate of mutagenesis on the dose of C^{12}

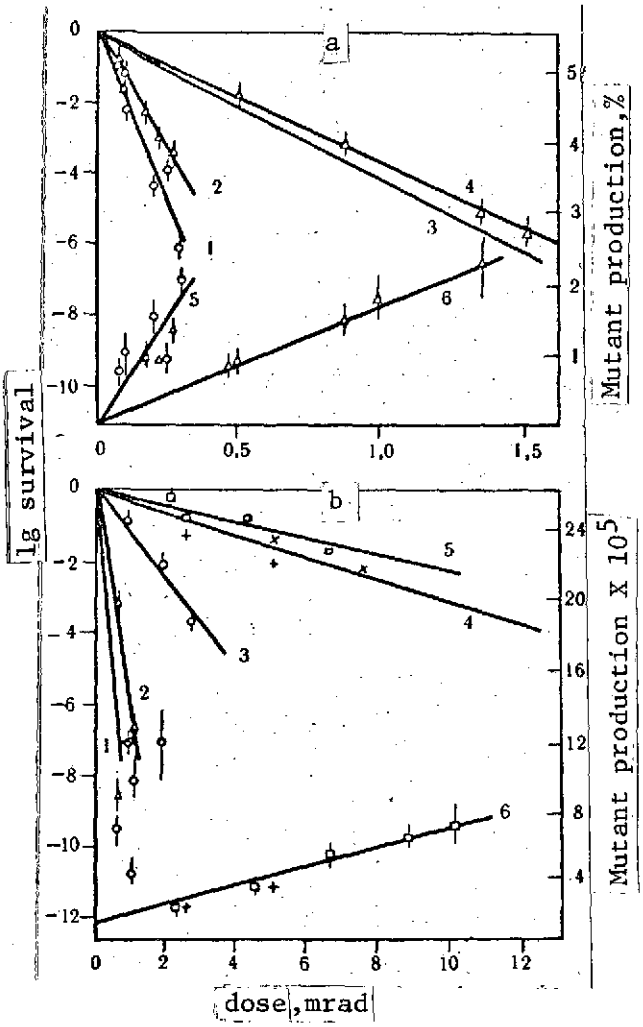

Figure 17. Inactivation and mutagenesis of phages C_D (a) and λ_{11} (b) during the effect of protons and γ-rays and heavy ions.

a — inactivation of phages C_D in bouillon during the effect of γ-rays (1), protons (2), dry specimens with the effect of γ-rays (3), dry specimens with the effect of protons (4), mutant production with the effect of γ-rays (0) and protons (Δ) on samples in bouillon (5), mutant production with radiation with protons and γ-rays of dry specimens (6);

b — inactivation of phage λ_{11} in bouillon with the effect of γ-rays (1), with the effect of protons (2), with the effect of γ-rays on dry specimens (3), with the effect of carbon ions on dry specimens (4), with the effect of neon ions on dry specimens (5), mutant production (6) with the effect of carbon (+) and neon (0) ions.

and Ne^{20} ions indicates the single-stroke mechanism of the development of virmutations in phage λ_{11}.

Thus, a common fact in the kinetics of mutagenesis of phages C_D and λ_{11} is their linearity for the tested range of doses, indicating single-stroke kinetics of the process. However, for phage C_D with survival on the order of 10^{-5}, a deviation from linearity is noted in the case of radiation with protons of dry specimens. The reliability of this fact can be proven, having only studied the behavior of mutation curves for lower levels of survival. It is noteworthy that the inactivating effect of heavy ions differs from that of /118

TABLE 28

RBE COEFFICIENTS FOR VARIOUS KINDS OF RADIATION

Kind of radiation	Energy of radiation Mev/nucleon	LET, Mev·cm^2/g	$1/D_{37}$ (10^{-4} rad^{-1})	P	RBE coefficient
γ-rays	-	3.0	1.65±0.04	-	1.0
H^{11}	645	2.4	1.51±0.05	0.05	0.91±0.04
B^{11}	7.3	1400	1.22±0.09	<0.01	0.74±0.05
C^{12}	5.8	2300	1.05±0.05	<0.001	0.64±0.04
B^{11}	1.5	3900	0.43±0.02	<0.001	0.26±0.02
Ne^{20}	8.4	5000	0.67±0.04	<0.001	0.41±0.02
Ar^{40}	1.5	3000	0.09±0.01	<0.001	0.07±0.01

protons and γ-rays. Survival of phages with various doses of heavy ions and, for example, γ-rays, is 4-5 times higher. Evidently, this is due to the uneveness of ionization produced by heavy particles in the irradiated volume. Although within the limits of the tracks the lethal effect is very strong, the degree of "utilization" of ions will, on the average, be several times lower.

Analysis of materials obtained in radiating bacteria E. coli B with heavy ions, as well as with γ-rays and protons, shows that the dose-effect dependence bears an exponential character. In comparing the biological effect of heavy ions and γ-rays, RBE coefficients were calculated. The effectiveness of γ-radiation was taken as one. RBE coefficients are given in Table 28. From these materials it is obvious that RBE of heavy ions are less than one and are reduced as LET of particles increases. The reduction of RBE as LET of particles increases reflects the different character of the microdistribution of absorbed energy in biological objects during the effect of heavy ions and slightly ionizing radiation and can indicate that passage of a heavy charged particle through an effective volume of cells is accompanied by release of energy greater than necessary to inactivate the object.

Thus, data on objects with different levels of biological organization indicate significant differences in radiobiological effects caused by heavy ions and slightly ionizing radiation. The biological effect of radiations with various ionization density depends on the microdistribution of the absorbed dose in the radiated objects.

N75 14418

The Effect of Synthetic Homopolymer Poly I:C on the Synthesis of Nucleic Acids, Protein and Interferon in Spleen Cells Normally and with Radiation

A group of studies has shown that polyinosinic-polycytidinic acid (poly I:C) and other synthetic double-stranded polyribonucleotides increase humoral and cellular immunological response [51, 67].

It has been established that synthetic ribonucleotide homopolymers stimulate antibody formation by increasing the proliferation of antibody-forming cells. However, the mechanism of the effect of synthetic ribonucleotide homopolymers is, evidently, not limited to activation of DNA synthesis and proliferation. It has been shown, for example, that the synthesis of interferon is activated under the effect of synthetic homopolymers after intervals of time too short for this increase to be explained by multiplication of producer-cells of interferon. /119

Data is absent on the sequence of changes in the metabolism of DNA, RNA and protein in the spleen cell. Despite known toxicity of synthetic homoribopolymers when administered to experimental animals, there is much practical interest in studying the prospects of using these preparations to restore specific and nonspecific immunity after the damaging effect of extreme factors, including radiation.

Taking the above into account, we conducted a comparative study of the effect of the synthetic homopolymer poly I:C and Newcastle Disease virus on the synthesis of RNA, DNA, total protein and interferon in the spleen of nonradiated and radiated mice.

We assumed that besides potentiation of the effect of the antigen on corresponding competent cells, synthetic homopolymers by themselves affect many

lymphoid tissue cells. This assumption was also tested on intact and radiated animals.

Tests were made on 200 inbred BALB/c male mice weighing 18-20 g.

As inducers of interferon-formation we used a lyophilized preparation of synthetic homopolymer poly I:C (Calbiochem, USA), which was injected in the tail vein of the mice in doses of 50 μg.

A suspension of Newcastle Disease virus (NDV-Hertfordshire strain) was also injected intravenously ($10^{9,0}$ LD_{50} per mouse).

Half the test animals were exposed to a single acute γ-radiation of 400 R. In 24 hours or 4 days after radiation the animals were injected with an inducer of interferon formation. The animals were killed in 1, 6 and 18 hours after administration of the inducer. A cellular suspension was prepared from spleens by rubbing through a kapron seive. After the cells were washed the suspension was reduced to a concentration of 40×10^6 cells per 1 ml and poured out into 0.5 ml batches for parallel tests.

As radioactively labelled precursors we used C^{14}-uridin (Czechoslovakia, specific activity 72 μ/mM), H^3-thymidine (England, specific activity 5 μ/mM) and C^{14}-labelled cholera protein hydrolysate (Czechoslovakia, specific activity 80 μ/mM) in final concentration of 1 μ/ml. Contact of the cells (20×10^6 cells) with labelled precursors lasted 60 minutes at 37°. Then the cells were washed twice with a cold medium. Suspensions of cells incubated with H^3-thymidine were studied radioautographically. For this we prepared smears and coated them with emulsion — M(NIKFI). After exposure at 0° the preparations were developed, stained with methyl green-pyronine and the proportion of lymphoid cells which included H^3-thymidine counted. In each preparation at least 2000 cells were analyzed.

Cells incubated with precursors labelled with C^{14} were precipitated in millipore filters (Chemapol), washed repeatedly with a chilled 5% solution of TCU and then with 80° ethanol. The radioactivity of filters was counted in a scintillation counter (Tricarb, USA). /120

Interferon was determined in serum and in a homogenate of spleen cells 1, 6 and 18 hours after the administration of inducers by suppression of the cytopathic effect of Semliki Forest virus (SFV). The titer of interferon was expressed as the highest dilution of the sample suppressing the cytopathic effect of SFV. Adsorption of macrophages was conducted according to the Forbes method by incubation of the cellular suspension with sterile wadding.

TABLE 29

THE EFFECT OF RADIATION AND THE ADMINISTRATION OF INTERFERON INDUCERS ON RNA SYNTHESIS IN SPLEEN CELLS OF BaLB/c MICE

Inducer of interferon	Synthesis of RNA, %		
	Hours after administration of inducer		
	1	6	18
Without radiation:			
without inducer	100*	---	---
poly I:C	100	160	160
NDV	130	115	160
Radiation			
Co^{60} (400 R)			
without inducer	30	---	---
poly I:C	20	24/13**	25/14**
NDV	25	18/14	20/11

*Radioactivity of suspensions including uridin-H^3 were expressed in pulses per minute per 10^6 cells; activity of spleen cells of intact animals was taken as 100%.

**In the numerator — RNA synthesis in comparison with non-radiated control animals without inducer, in the denominator — in comparison with the administration of the inducer to non-radiated animals at the same time.

In Table 29 are given the results of a study of RNA synthesis (inclusion of H^3-uridin) in spleen cells of radiated and non-radiated mice after the administration of interferon inducers.

During the first hour after the administration of poly I:C to non-radiated mice, RNA synthesis in spleen cells did not differ from the control level. In 6 and 18 hours a significant activation of RNA synthesis (60%) was noted in spleen cells.

Different results were obtained in analogous tests on radiated mice. As shown in Table 29, radiation reduced RNA synthesis in the spleen 3 times (scaled to one cell). After the administration of poly I:C, RNA synthesis in spleen cells of radiated animals was practically unchanged. Thus, in radiated animals, unlike intact mice, poly I:C had no stimulating effect on the synthesis of RNA in spleen cells.

The synthesis of total protein (inclusion of amino acids labelled with C^{14}) in non-radiated mice after the administration of poly I:C did not change (Fig. 18, a). After radiation the synthesis of protein decreased an average of 30° (Fig. 18, b). The administration of poly I:C led to a further slight reduction in the intensity of synthesis.

The calculation of cells including H^3-thymidine taken from non-radiated and radiated animals showed no increase in the proportion of cells synthesizing DNA 2, 6 and 18 hours after administration of the inducer.

The results of a study of the effect of Newcastle Disease virus on the synthesis of nucleic acids and protein in spleen cells of mice are given in Fig. 19, a. From this illustration can be seen that activation of RNA synthesis in non-radiated animals was already observed an hour after the administration of the inducer and the rate of inclusion of H^3-uridin remained at a comparatively high level for at least 18 hours. /121

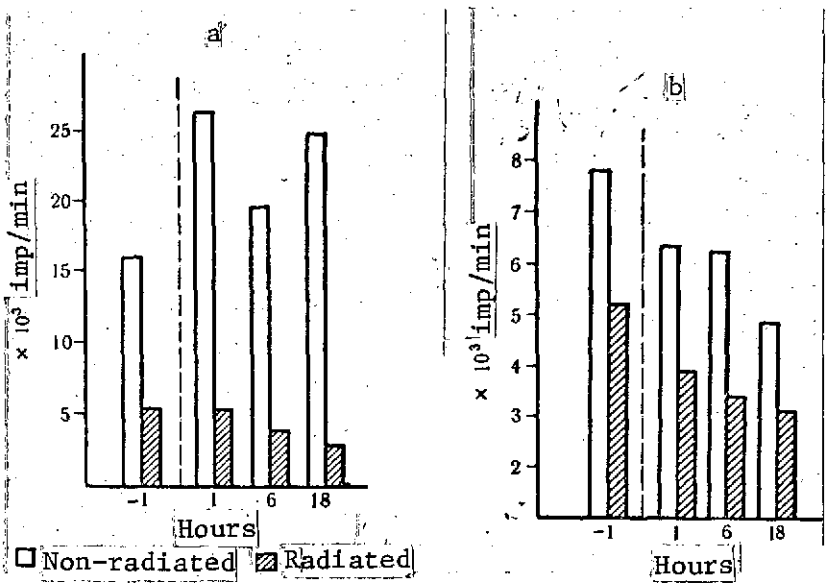

Figure 18. Rate of RNA synthesis according to the inclusion of C^{14}-uridin (a) and protein synthesis by the inclusion of C^{14}-amino acids (b) in spleen cells of mice, non-radiated and radiated once in doses of 400 R before administration and at different times after administration to the animals of synthetic polynucleotide.

TABLE 30

THE EFFECT OF RADIATION AND THE ADMINISTRATION OF INDUCERS ON THE INTERFERON CONTENT IN BLOOD SERUM OF MICE

Inducer of interferon	Titer of interferon*		
	Hours after administration of inducer		
	1	6	18
Without radiation:			
without inducer	2	2	2
poly I:C	320	160	8
NDV	16	640	32
Radiation Co^{60} (400 R):			
without inducer	2	2	2
poly I:C	320	160	4
NDV	4	320	16

*In 0.5 ml of blood serum taken from 6 mice.

In radiated animals there was no increase in the synthesis of RNA after administration of NDV.

The rate of protein synthesis in spleen cells of non-radiated animals after the administration of the inducer did not change; when the inducer was combined with radiation, a reduction was observed in the rate of inclusion of C^{14}-amino acids in spleen 18 hours after the administration of Newcastle virus /122 (Fig. 19, b).

As seen from Table 30, cobalt radiation in doses of 400 R had no effect on the formation of circulating interferon induced by the administration of poly I:C. Interferon was formed at the same times and in comparable amounts in radiated and non-radiated animals. Some reduction in the titer of interferon was noted in radiated animals when Newcastle Disease virus was used as the inducer of interferon. These results agree with our earlier data concerning the effect of x-radiation on the formation of interferon under the influence of poly I:C [88]. The induction of interferon caused by Newcastle Disease virus was sensitive not only to x-rays, but as our tests show, also to γ-radiation.

A study of cellular composition in suspensions of spleens of radiated and non-radiated mice showed that γ-radiation in doses of 400 R after 4 days caused a significant decrease in the content of lymphoid cells. The administration to non-radiated mice of poly I:C after 18 hours caused a slight increase in the proportion of lymphoid cells; Newcastle Disease virus did not change the cellular composition of the spleen.

The administration of both inducers to radiated mice did not significantly affect the content of lymphoid cellular elements in the spleen in the next 18 hours.

In Table 31 are given the results of a study of the interferon content in cells of mouse spleen before and after adsorption according to the Forbes

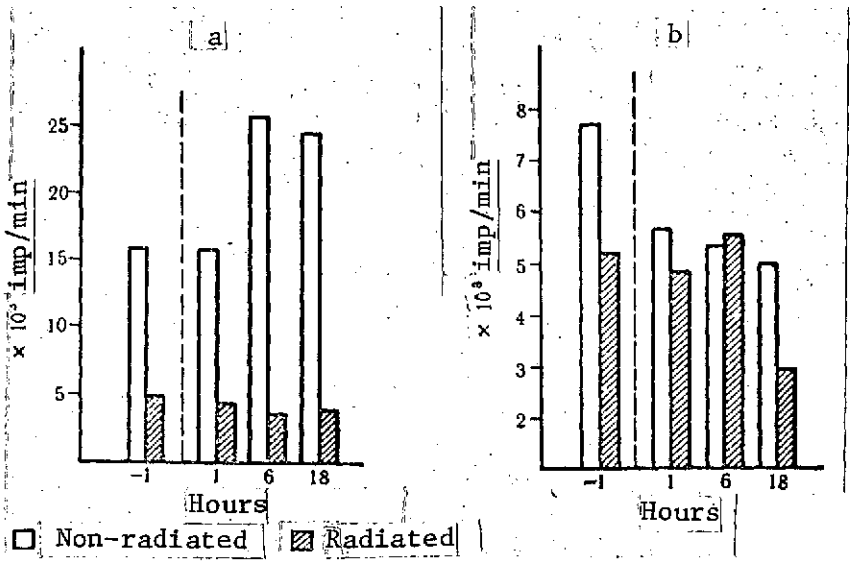

Figure 19. Rate of the synthesis of RNA (a) and protein (b) in spleen cells of mice, non-radiated and radiated once with doses of 400 R before the administration and at various times after the administration of Newcastle Disease virus.

method. These data show that adsorption does not affect the induction of interferon by the administration of poly I:C. However, titers of interferon in these tests are too low to consider the result completely reliable. With the administration of NDV, removal of macrophages from the cellular suspension caused practically a complete disappearance of interferon.

The stimulating effect of nucleic acids and nucleotides on lymphocytes /123
and the synthesis of antibodies has been discussed most thoroughly in the series of works by Braun et al. [51, 52] and Johnson et al. [67, 68]. We showed the activating effect on cell proliferation in lymphoid tissue and on the secondary immunological response of RNA, ribonucleotides and RNA-ase in vivo and in vitro [23].

TABLE 31

SYNTHESIS OF INTERFERON BY SPLEEN CELLS OF BaLB/c MICE UNDER THE EFFECT OF POLY I:C AND NEWCASTLE DISEASE VIRUS

Treatment* of mice	Adsorption** of cell suspension	Hours after administration of inducer			
		0	1	6	18
Without treatment	Before	2	-	-	-
	After	2	-	-	-
Co^{60}	Before	2	-	-	-
	After	2	-	-	-
Poly I:C	Before	-	2	4	2
	After	-	2	4	2
Co^{60} + poly I:C	Before	-	8	16	4
	After	-	8	16	4
NDV	Before	-	8	16	4
	After	-	2	2	2
Co^{60} + NDV	Before	-	16	32	4
	After	-	2	2	2

*Co^{60} – mice radiated with γ-rays of Co^{60} in doses of 400 R; inducers poly I:C and NDV administered on 4th day after radiation

**Titers of interferon were determined in 0.5 ml of supernatant liquid obtained after centrifuging homogenate from 8×10^6 cells before or after adsorption.

It can be assumed that nucleotides and polynucleotides can affect the cell in two ways: by reutilization of nucleotides for the synthesis of its own nucleic acids or by the effect on regulator systems of the cell. Of the latter there is special interest at present in the adenylate cyclase system of the lymphocyte (see below).

No doubt some single-chain synthetic homopolymers act in the second way, as increase in the content of one ribonucleoside-monophosphate is not able to create the necessary reserve of precursors for the synthesis of nucleic acids.

The effect of double helix homoribonucleotides, observed in vivo, can also be achieved in vitro by using single-strand homopolymers and oligonucleotides. By protecting single-strand polymers from RNA-ase by complexing

with methylated BCA a distinct stimulating effect can be obtained in vivo.

Braun et al., [52] expressed the opinion that the essential link in the effect of synthetic homoribopolymers on the lymphocyte is adenylate cyclase — an enzyme transforming adenosine triphosphate into cyclic adenosine monophosphate. The latter, according to the data of a number of authors, is the main activator in the chain of processes leading to cellular proliferation in lymphoid and homopoietic tissue.

The stimulating effect of homopolymers is removed by chloramphenicol, i.e., it is needed in the synthesis of any proteins.

Studies carried out up until now have shown that components of poly I:C penetrating the cell do not act as matrix RNA. True, there are individual re- /124
ports that poly A:U stimulates the inclusion of phenylalanine.

Under our test conditions, the administration of poly I:C to mice caused comparatively early activation of the synthesis of total RNA in the spleen cells. No increase in the proliferation of cells (by the inclusion of H^3-thymidine) or acceleration of synthesis of total protein could be established. It is not ruled out that the synthesis of some kind of protein, not caught in summary analysis, was activated and was necessary for the new formation of large amounts of RNA. However, it is evident that a significant increase in the synthesis of ribonucleic acids was not connected at early points with providing cellular proliferation and did not cause significant acceleration of the synthesis of many proteins of the splenetic cell.

Collavo et al., showed that poly I:C causes proliferation in the mouse spleen of many cellular populations; hyperplasia of myeloid and erythroid cells and megakaryocytes was observed.

It can be assumed that early changes in RNA synthesis, which we described, are one of the first metabolic steps in the process leading later to proliferation of lymphoid and other cells of the spleen. Evidently, this ac-

tivation of total RNA synthesis in spleen cells reflects the total activation of transcription processes necessary to accelerate the production of various-quality proteins.

Changes in metabolism were studied comparatively soon after the administration of poly I:C. It can be assumed that this activation of RNA synthesis is the first step for later intensification of proliferation of T-lymphocytes in the spleen.

This also relates to a certain degree to the effect on spleen cells of Newcastle Disease virus, although a distinct acceleration in the synthesis of RNA in non-radiated mice in these tests occurred only an hour after administration of the virus.

In tests, in vitro activation of RNA synthesis was observed in peritoneal macrophages, caused by homopolymers (poly A:U). The possibility of such an effect of poly I:C on macrophagal elements of the spleen in vivo must be kept in mind.

Poly I:C and Newcastle Disease virus activate the synthesis of interferon in intact animals. There is interest in the paradoxical effect of double-chain homopolymers on the synthesis of RNA and interferon in spleen cells of radiated mice. While reduction of RNA synthesis, caused by radiation, also increases slightly under the effect of poly I:C and the virus, the synthesis of interferon in spleen cells and in the entire body (titers in serum) is activated.

It is known that activation of the synthesis of interferon requires an increase in DNA-dependent synthesis of RNA in the cell. It is assumed that the effect of poly I:C on the synthesis of antibodies, interferon and the activity of the reticuloendothelial system is based on similar mechanisms. It is difficult, therefore, to assert that in our tests the combination of radiation and homopolymers of ribonucleotides in the same cell inhibit the syn-

thesis of RNA, but against such a background the synthesis of interferon RNA is activated and the necessary concentration of RNA provided as well as ribosomes in cytoplasm necessary for RNA to read this. More probable is the assumption that the target for poly I:C and the site of activation of interferon synthesis in the spleen of a radiated mouse is cells resistant to radiation. According to the data of several authors, such resistant elements are cells with a macrophagal function. They withstand radiation in doses of 10,000 R and thereafter maintain the ability to interact with T- and B-lymphocytes in /125
the biosynthesis of antibodies in the spleen.

As is known, macrophages are one of the cellular elements which are "suppliers" of interferon. The assumption that in the mouse the basic (if not the only) splenetic cell producing interferon is the macrophage required experiments with fractionation of spleen cells after radiation and the administration of an inducer.

Unclear results were obtained in tests with poly I:C, possibly because of the low titer of interferon in the initial population. In tests with Newcastle Disease virus, however, removal of macrophages from the cell population of the spleen caused a significant reduction in the titer of interferon.

Further deeper analysis of this question is of special interest, as it makes it possible to speak of preservation after radiation in the radioresistant cell of the entire system of biochemical reactions mediating the induced synthesis of protein. This, in turn, will necessitate raising the question of the existence of a close connection (or even common character) between "molecular targets" for ionizing radiation and synthetic homoribopolymers and the uniqueness of such a target in highly radiosensitive cells, for example, lymphocytes.

Individual Radiosensitivity and its Daily Variations

Radiosensitivity of the body of animals is one of the important and complex problems in modern radiobiology. In evaluating it, it is necessary to take into account a number of factors determining the reaction of the body to ionizing radiation: species, variety, age, sex and weight of the animals; the functional state of the body and its systems at the moment of the effect, which depends on environmental conditions — season, temperature, time of day, etc. But even with uniformity of all these conditions, the reaction of individual animals to radiation in the same dose is not the same and here we are dealing with individual radiosensitivity of the body.

At the present time there are two approaches to preliminary evaluation of individual radiosensitivity of the body: determination of ratios between the initial state of the body and its radiosensitivity and establishment of radiosensitivity of the body on the basis of evaluation of its reactivity [11].

Studies in the first direction established the following. L. Novak et al. [76] recommend using as a criterion for determining individual radiosensitivity deviation in the consumption of oxygen by a given animal from average group values of oxygen consumption or calculated values of a "biophysical model," taking into account a number of factors of the external and internal environment.

Deviation from average group values of the amount of fluid excreted per day is also suggested for evaluation of the probability of death of the animals at different times after radiation [4]. Mitotic activity of skin cells of intact animals can also be used to predict the outcome of radiation injury.

More justified are examples of preliminary evaluation of individual radiosensitivity by means of studying the function of the system determining the outcome of radiation injury of the body in the tested range of doses. The at- /126

tention of a number of researchers has been directed toward individual differences in the activity of the hematopoietic system in comparison with reactions to radiation in doses causing a hematopoietic form of radiation injury. Karpfel and Soska [69] turned their attention to the possiblity of evaluating the radiosensitivity of individual bodies by the preservation of leukocytes in peripheral blood. Besides this, I. A. Nechayev et al., [35] suggest using proteinase activity of the blood and the reaction of blood sugar to the administration of epinephrine. The difference in $LD_{50/30}$ in rats standardized according to total evaluation of the above indices was 30-70 R.

The research of Karpfel et al., [70] found that in individuals with a high level of spontaneous aberrations in marrow cells, after γ-radiation the marrow has less proliferative capacity and forms fewer spleen colonies after its administration to recipients.

Le Suan Tu and Ye. Ya. Grayevskiy [31] established individual differences in the content of endogenic sulfhydryl compounds in the bone marrow of rats. The level of sulfhydryl groups, primarily protein, in individual animals correlates with their resistance to the subsequent radiation effect.

Besides the above, there are a number of other methods of determining individual radiosensitivity by the reactivity of the body.

Individual differences in dogs can be predicted with the help of a physical or thermal load. Animals with a pronounced adaptative reaction of cardiovascular, respiratory and nervous systems are more resistant to radiation. "Unstable" animals, which have a strong or, just the opposite, a very weak reaction, can be considered radioresistant.

N. A. Vasil'yev et al., [5] found that increase in the consumption of oxygen during the brief effect of cold stress is a favorable prognostic sign [7].

Pospisil et al., [80, 81] showed that the variability of the Na/K ratio in the urine of animals kept under isolation and starvation conditions can be used to predict post-radiation survival, the development of leukopenic reaction and reduction in the weight of the body and thymus. Individuals with low and high mobility of this index were designated as "unstable" types. High mortality and sharper loss of weight after radiation were observed among this group of animals. An analogous correlation was found in studying the reaction of leukocytes in peripheral blood to starvation and isolation. A.S. Khil'ko et al., [46] suggested using the degree and direction of the reaction of leukocytes in peripheral blood to the administration of epinephrine to evaluate individual radiosensitivity. The leukocyte reaction of animals, in the opinion of the authors, was considered as a positive prognostic sign.

One of the aims of our research was to compare the effectiveness of determining individual radiosensitivity by several of these methods which could be used to examine rather large groups of animals (rats). The following methods were chosen for approval: the difference in oxygen consumption (ΔO_2) from average group values, determination of the initial content of leukocytes in peripheral blood ΔNa/K [80, 81] and the reaction of leukocytes to the / 127 administration of epinephrine.

The following changes were made in these methods:

— in approving the Novak method total gas exchange in rats was tested by the chamber method on a Breslav machine for 30 minutes;

— study of ΔNa/K in rats was carried out by a method modified to suit the purposes of this study. For 5 days the rats were kept individually in exchange cages; on the 1st, 3rd and 5th days they were deprived of food. Every day the urine of the animals was collected and its content of Na and K determined by the flame photometry method, after which the difference (Δ) in the ratio of excreted amounts of Na and K under repeated stress conditions was found [81]. Individual reactions under such conditions were quite pronounced. However, the range of variations in this index and its prognostic significance

for evaluating the degree of radiation damage changed essentially according to the season of the year;

— the reaction of leukocytes with intra-abdominal administration of epinephrine (0.25 mg/kg) was studied an hour (according to the method suggested by Khil'ko 2 hours after subcutaneous injection) later, as according to our data

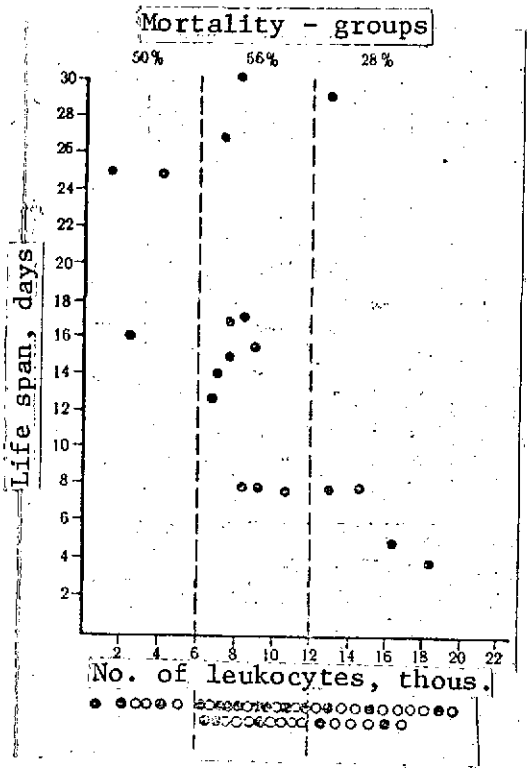

Figure 20. Life span and mortality in individual groups of rats (with different initial leukocyte contents in peripheral blood) after acute radiation in doses of 625 R.

Black circles — dead animals; white — those surviving for 30 days. Above is given mortality of the groups.

at such times the most significant individual differences in reaction are observed.

An experimental study was conducted on 600 male Wistar rats weighing 160-200 g in a light regime of 12 hours of light and 12 hours of darkness at a temperature of 22±1°. After examination by the previously indicated methods they were radiated with γ-rays of Co^{60} in doses of 625 R; dose rate was 396-428 R/min. Life span of the animals and mortality in individual groups was

determined for 30 days of observation. Results of the study are given in Fig. 20-22, b. /128

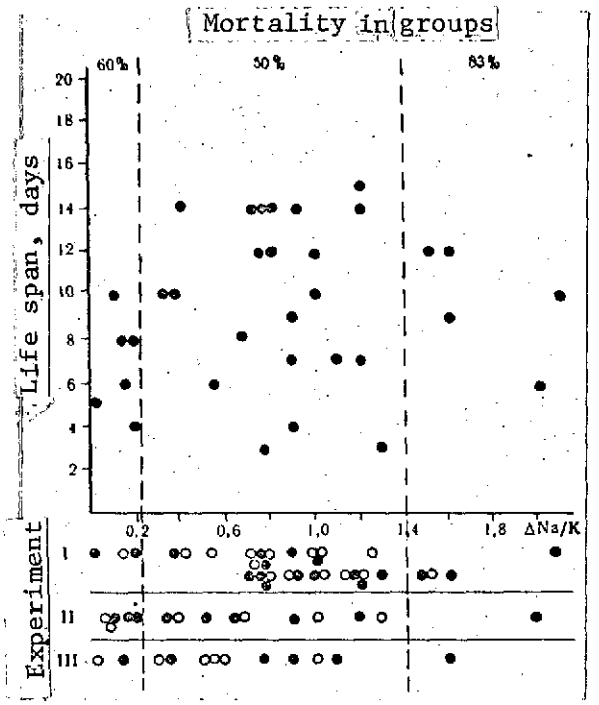

Figure 21. Life span and mortality in individual groups of rats (with different initial indices of ΔNa/K in urine) after acute radiation in doses of 625 R.

I, II, III — individual experiments. Other symbols same as in Fig. 20.

The most indicative results of predicting individual reaction to radiation were obtained by the leukocytic reaction to epinephrine load, ΔNa/K under repeated stress conditions and the initial content of leukocytes in peripheral blood. These approaches were used to determine both the probability of death of the animals in the 30 day period and their life span.

The difference in survival of individual groups of animals, selected according to leukocytic reaction to the administration of epinephrine, reached 52% (see Fig. 22, b), according to ΔNa/K — 33% (see Fig. 21) and according to initial level of leukocytes in peripheral blood — 28% (see Fig. 20). In these studies we were not able to detect a correlation between ΔO_2 and post-radiation survival and the life span of the animals.

Further experimental development of the matter concerning individual differences in radiosensitivity was carried out using the method of standardization (a group of individuals similar in radiosensitivity) according to the reaction of leukocytes in peripheral blood to the administration of epinephrine. In examining experimental animals between 9 to 12 a.m., 16.2% of the entire population was composed of individuals with a pronounced leukocytic reaction ($\Delta L < +5000$). This group of animals was arbitrarily designated as radioresistant (see Fig. 22 a and 22 b), unlike the average of the group ($-4000 < \Delta L < +5000$) — radiosensitive; such individuals are the majority of the population — 73% (see Fig. 22 a). Because of insufficient experimental data we are still not able to determine the radiosensitivity of the group giving a pronounced leukopenic reaction ($\Delta L < -4000$). Difference in $LD_{50/30}$, calculated according to the Litchfield-Wilkinson method, between the radioresistant group and the usual population sample, where the majority are radiosensitive, reaches 100 R (Table 32).

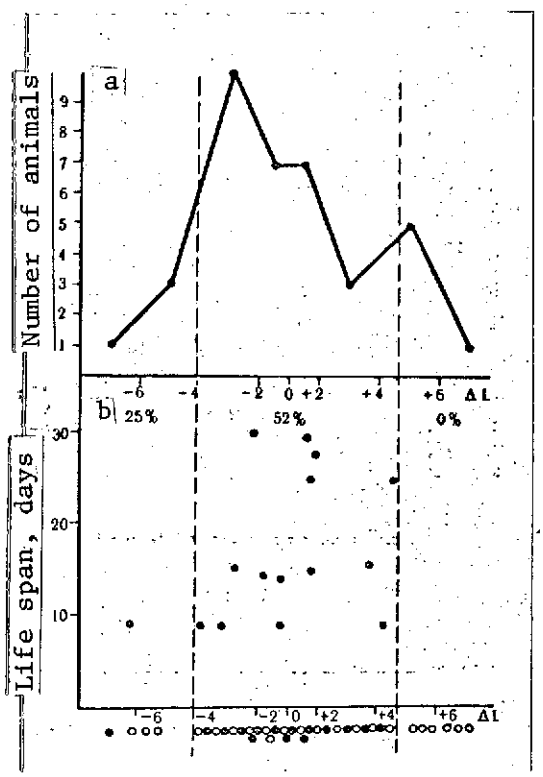

Figure 22. Number of animals in groups with various leukocytic reactions in the epinephrine test (a); life span and mortality in individual groups of rats (with different initial leukocytic reactions ΔL) in epinephrine test after acute radiation in doses of 625 R (b). The other symbols are the same as in Fig. 20.

Therefore, the selected method of standardizing rats is more effective than that suggested by I.A. Nechayev et al. [35].

An attempt has also been made to reveal the difference in composition of the pool of stem cells between radiosensitive and radioresistant groups of rats by xenogenic transplantation of bone marrow cells to CBA mice [21]. Splenetic colonies (KOE) were formed in large number with the administration of bone marrow cells of radioresistant rats (Table 33). These results support the report of Karpfel [70] of the existence of individual differences in the pool of stem cells (which is more probable) or their proliferative activity.

TABLE 32

CALCULATED $LD_{16/30}$, $LD_{50/30}$ AND $LD_{84/30}$ FOR WISTAR RATS

LD	Ordinary sample, rad	Radio resistant group, rad
16/30	424	575
50/30	624	730
84/30	828	875

TABLE 33

NUMBER OF COLONIES IN THE SPLEEN OF CBA MICE WITH THE ADMINISTRATION OF BONE MARROW CELLS OF RATS (M±m)

Number of injected cells	Radio sensitive rats	Radio resistant rats
$1 \cdot 10^6$	2.5±0.4	8.0±1.2
$5 \cdot 10^6$	10.1±0.45	13.8±1.0

Later our attention was attracted to the essential reduction in the range of individual variations with a prolonged effect of ionizing radiation. /130

The index of individual variations of radiosensitivity $I = \left(\frac{LD_{84/30} - LD_{16/30}}{LD_{50/30}} \right)$ in daily radiation is reduced 6 times (I = 0.11) in comparison with acute radiation (I = 0.65). This fact, in comparison with numerous data on the existence of daily changes in the pool of stem and fissionable cells, the morphological state of organs of hematopoiesis [44], daily changes in radiosensitivity of both the whole body and the hematopoietic system gives basis for assuming that individual differences in radiosensitivity during acute radiation are basically due to dissimilar changes during the course of the day in

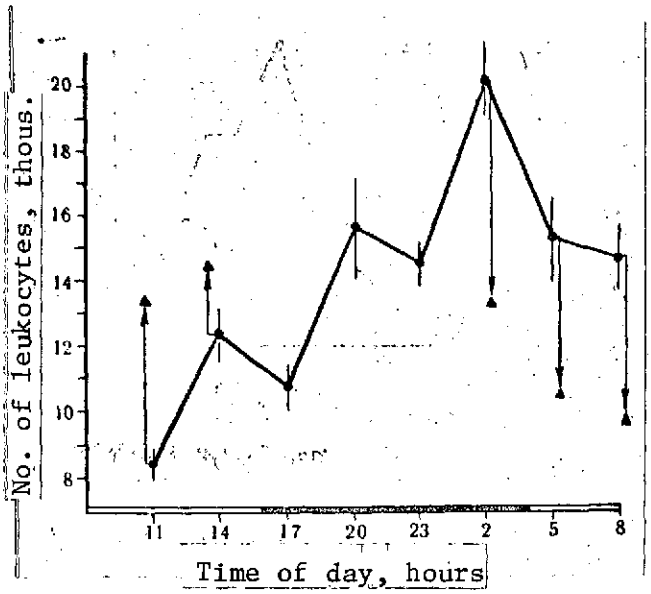

Figure 23. Change in the content of leukocytes in peripheral blood during the day (circles) and their reaction in the epinephrine test (triangles).

individual bodies in the hematopoietic system, particularly, in links determining the outcome of the radiation effect.

This assumption impelled us to approach methodological details of radiosensitivity standardization of rats more attentively. It was found that the direction of changes in the leukocyte content of peripheral blood after the administration of epinephrine depended on the initial level during the day (Fig. 23) and conformed to Wilder's law of initial values. In the epinephrine test, animals with a low leukocyte content in peripheral blood revealed a predominance of the leukocytic type of reaction during the second half of the light period (11 to 14 hours); with a high initial content of leukocytes (in the second half of the dark period and beginning of the light period) — a leukopenic reaction predominated. Therefore, in conducting experimental works on individual radiosensitivity it is necessary to know definitely and take into account the daily periodicity in the function of body systems. But even with studies conducted at the same time of the day (9-12 hrs.) on animals synchronized for 3-4 weeks under an artificial light regime, we encounter sig-

nificant variability in both the initial content of leukocytes in peripheral blood and in its change in the epinephrine test (see Fig. 22, a). /131

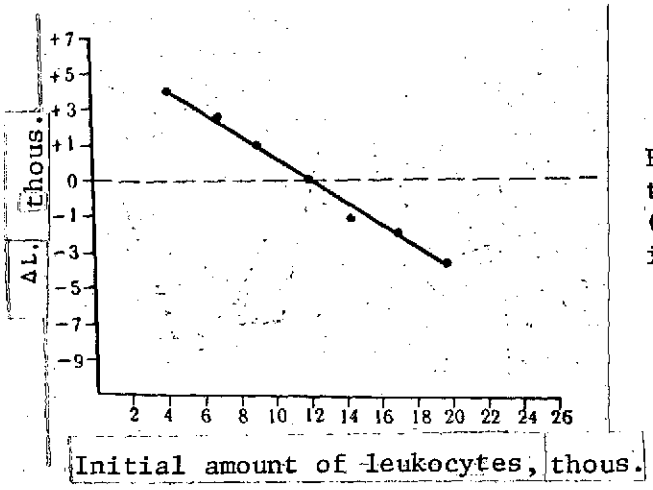

Figure 24. Dependence of the reaction of leukocytes in peripheral blood (in the epinephrine test) on their initial level.

Analysis of data on the epinephrine test in animals in the morning revealed a distinct dependence of the reaction of leukocytes on their initial level (Fig. 24). A null reaction in the epinephrine test is observed with an initial leukocyte content in peripheral blood of about 12 thousand per 1 mm^3. This is close to the daily mean level of the leukocyte content (see Fig. 23). The slight difference can be due to seasonal variations. Therefore, later data are a verification of the hypothesis that individual differences in the reaction to acute radiation are caused by differences in the course of periodic processes of the body, in rhythms of reproduction of cells in "critical" systems. However, further studies are necessary for final justification of this assumption.

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Daily Rhythms of Radiosensitivity of Animals and Several Determining Causes

The sensitivity of the body of animals to the effect of ionizing radiation is determined by the state of its critical systems. However, periodic changes in functional activity in relation to geophysical cycles are typical of the majority of systems of the body.

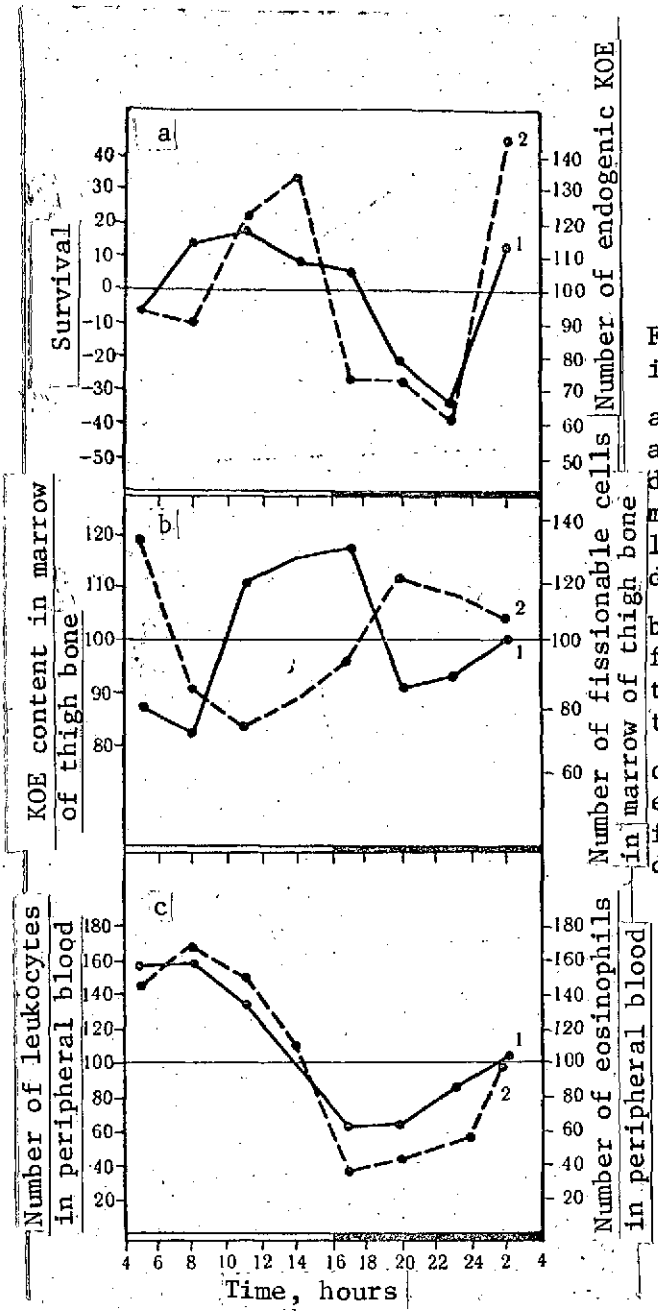

Figure 25. Daily changes in individual indices in CBA mice. /132

a — survival of mice (difference from average daily level) after radiation dose of 675 R (1), endogenic clone formation (in percentages of average daily level) on the 10th day after radiation dose of 600 R (2).

b — KOE content (1) and number of fissionable cells (2) in marrow of the thigh bone of intact mice (in percentages of average daily level).

c — content of leukocytes (1) and eosinophils (2) in peripheral blood of intact mice (in percents and average daily level).

Pizzarello et al. [79] first turned the attention of researchers to the existence of daily rhythms in the sensitivity of animals to radiation. Later experimental study of daily variations in the radiosensitivity of a number of plant and animal objects gave contradictory results.

In the present chapter are given the principle results of research which we conducted at the Institute of Medico-biological Problems, USSR Ministry of

Public Health, and the Institute of Biophysics, Czechoslovakian SSR Academy of Sciences, studying the regularity of daily changes in radiosensitivity and causes of daily fluctuations in the radiation effect with doses causing primary damage to the hematopoietic system.

Random bred mice and rats, CBA, C57BL, C57BL/6, BALB/c, H and BFU mice and F_1 hybrid mice (CBA×C57BL/6) were used in the studies. The animals were kept under constant temperature conditions in a light regime of 12 hours of light and 12 hours of darkness with free access to food or feeding at the start of the active dark period of the day.

The creation of such conditions and determination of the optimum adaptation time to it by experimental animals has great significance for a careful study of periodic processes. This more or less completely synchronizes the circadian rhythms of processes of both the individual body and the entire population with external time clocks, primarily the light regime. /133

These requirements must also be taken into consideration in studying rhythms of radiosensitivity when, strictly speaking, daily changes in individual radiosensitivity must be methodically studied by effectiveness of radiation at different times of the day on individual samples of animals from a given population.

After 10-14 days under the above conditions, in experimental animals are established distinct single-period rhythms of general motor activity, total gas exchange, body temperature, leukocyte content in peripheral blood, proliferative activity of bone marrow cells, intestinal epithelium, epithelium of the cornea, etc. [13, 92, 93]. With more prolonged adaptation in random bred mice, CBA and C57BL/6 mice daily rhythms in the content of karyocytes could be found in bone marrow.

Study of daily rhythms of radiosensitivity began with determination of the survival of animals after acute total radiation in the same dosage at dif-

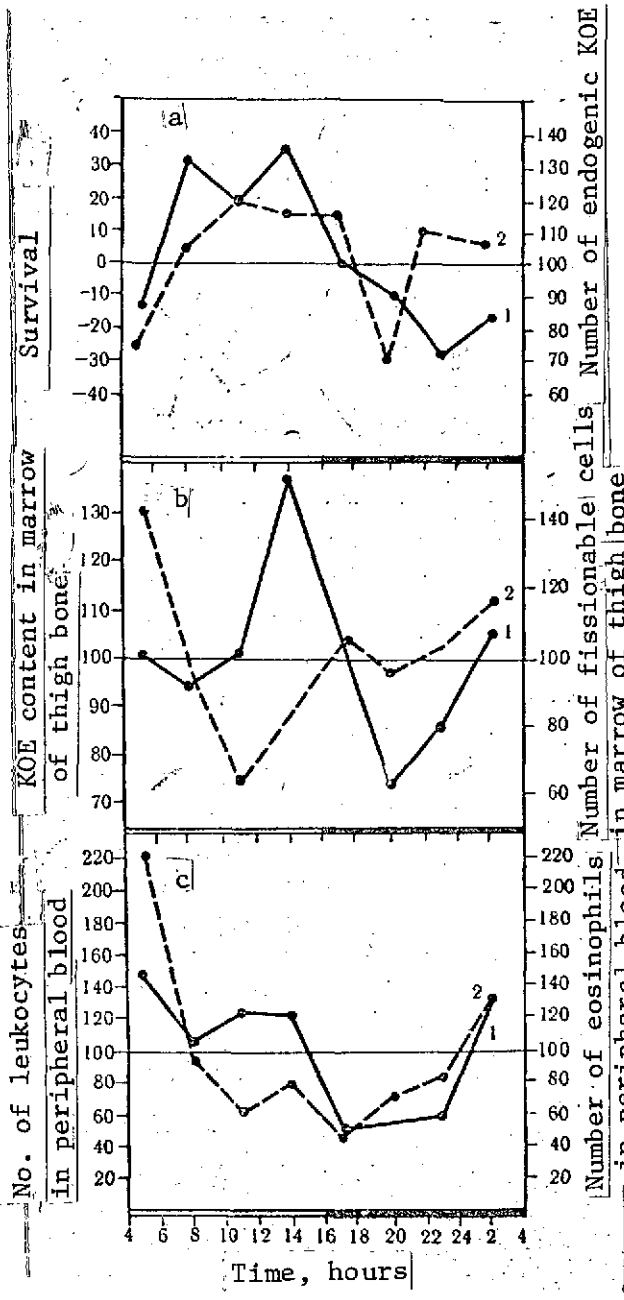

Figure 26. Daily changes in individual indices in C57BL/6 mice

a — survival of mice (1) and number of endogenic KOE (2) after radiation dose of 660 R; b and c — symbols same as in Fig. 25.

ferent times of the day. The course of the daily curve of radiosensitivity /134
according to the criterion of survival differs in animals of different species and varieties. The radiosensitivity of random bred rats, as that of random bred mice, usually doubled during the day. Rises in the radiosensitivity of rats basically coincide in time with changes in the light regime: from light to dark and from dark to light. Radiosensitivity of random bred mice increases in the middle of the light and dark periods. Maximum differences in

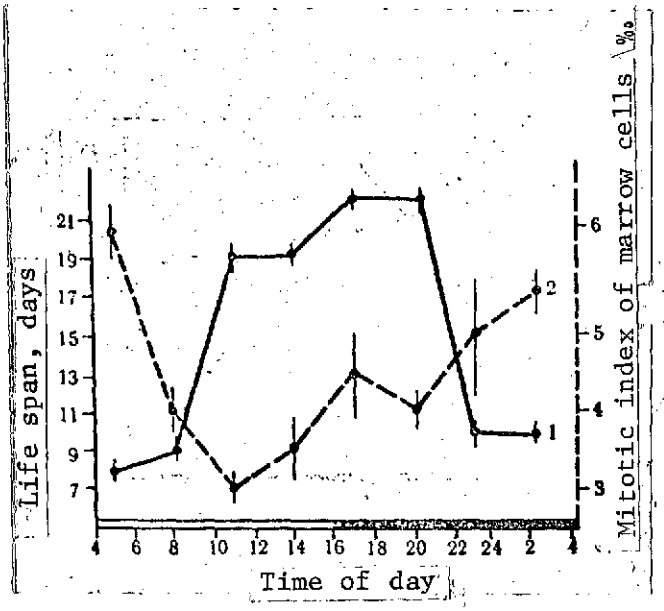

Figure 27. Dependence of the life span of CBA mice (dose of 1400 R) on the time of the radiation effect (1).

Daily fluctuations in mitotic activity of cells of the bone marrow of intact CBA mice (2).

TABLE 34

SURVIVAL OF C57BL/6 MICE AFTER ACUTE γ-RADIATION IN A DOSE OF 660 RAD AT DIFFERENT TIMES OF THE DAY

Time of radiation, hr.	Test 1, December				Test 2, May			
	Number of animals			survival, %	Number of animals			survival, %
	total	died	survived		total	died	survived	
05	20	11	9	45	20	6	14	70
08	20	6	14	70	20	1	19	95
11	20	3	17	85	20	0	20	100
14	33	15	18	54	20	0	20	100
17	20	9	11	55	20	1	19	95
20	20	15	5	25	20	6	14	70
23	20	20	0	0	20	6	14	70
02	20	8	12	60	20	0	20	100

$\chi = 39.47$ $f = 7$ $P << 0.01$

$\chi = 27.2$ $f = 7$ $P < 0.01$

Light regime: 12 L + 12 D, light turned on at 4.

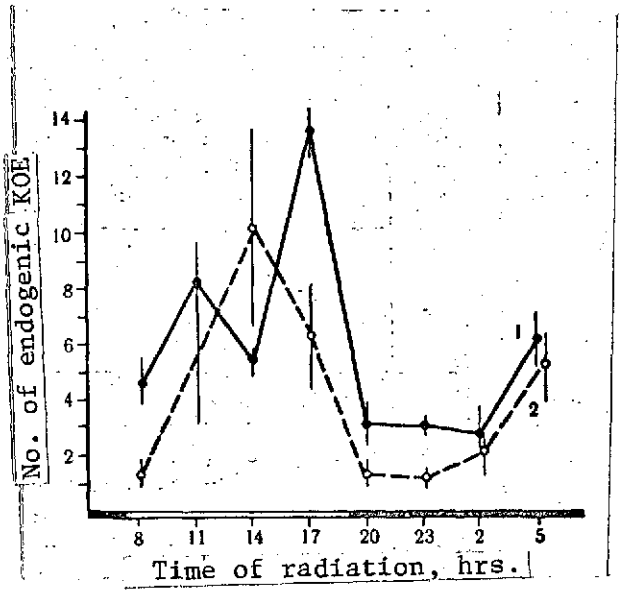

Figure 28. Number of endogenic colonies in the spleen of BALB/c mice (1) and H (2) after radiation at different times of the day.

the mortality of the animals, radiated at the same dose at different times of the day, usually do not exceed 50% [6, 13, 14].

In experiments on inbred mice we found both one and two increases in radiosensitivity during the day. The rise in radiosensitivity most often and in the majority of tested inbred mice coincides with the dark, active period of the day (Table 34, Fig. 25, a and 26, a). However, in male C57BL mice, according to the data of A. Batsek, greater mortality is observed with radiation in the light period. The difference in the mortality of inbred mice, radiated at different times of the day with the same dose, reaches 85% (Table 34).

When the animals were kept under constant light regime conditions of 12 hours of light and 12 hours of darkness, daily variations in radiosensitivity were also affected by longer cycles, particularly annual cycles. The effect of the season of the year on daily periodicity was noted in experiments conducted on random bred rats and CBA mice. In random bred rats the greatest rise in radiosensitivity during the autumn and winter comes at the end of the light and the start of the dark period, in spring and summer at the end of the dark and start of the light period [6]. Daily variations in radiosensitivity in CBA mice are clearly expressed in autumn and winter but not observed in spring [32].

To reveal daily variations in radiosensitivity the selection of dosage and criteria for evaluating radiation injury is very important. Data characterizing the dependence of mortality among mice on radiation dosage were analyzed according to the Litchfield and Wilkinson method. In CBA mice daily variations in the radiation effect according to the criterion of mortality in a 30-day period are reduced as radiation dosage is increased. In the winter the difference between $LD_{16/30}$ with radiation at 11-22 hours and 19-20 hours was 100 R, the difference in $LD_{50/30}$ — 53.5 R; no difference in $LD_{84/30}$ was found.

In springtime the daily differences in post-radiation mortality of CBA mice are essentially evened out [14].

In random bred rats differences in survival with radiation at different times of the day are increased as the radiation dose is raised. Difference in $LD_{50/30}$ was 100 R [14]. /138

If the greatest radiosensitivity of CBA mice according to the criterion of survival for 30 days is observed in the middle of the dark period at 23 hrs (see Fig. 25, a), in evaluating the effectiveness of radiation on the life span of the animals the increase coincides with the second half of the dark and the first half of the light period (Fig. 27).

Daily changes in the radiosensitivity of animals are basically due to periodic changes in the state of stem hematopoietic cells. This can be judged on the basis of the similarity in curves showing the dependence of survival of the mice and the formation of endogenic colonies in their spleens (KOE) on the time of radiation (see Fig. 25, 26, 28). Maximum formation of endogenic KOE and the highest survival rate of CBA and C57BL/6 mice usually occur in the middle of the light period, minimums of both indices in the middle of the dark period [14, 15]. An analogous dependence is established in examining BFU and H mice [84, 93, 94]. Such an effect was not observed in mice kept under the same experimental conditions, but with natural alternation of light and dark [93].

TABLE 35

/136

DAILY CHANGES IN SEVERAL INDICES OF THE STATE OF THE HEMATOPOIETIC SYSTEM IN CBA MICE

Tested indices	Time during day of study, hr.								Average daily level
	05	08	11	14	17	20	23	02	
Formal elements of peripheral blood, thous. per 1 mm^3:									
leukocytes	18.5± ±1.9	13.3± ±0.8	15.6± ±1.4	15.4± ±0.8	6.4± ±2.7	6.9± ±0.3	7.4± ±0.3	16.6± ±1.2	12.5± ±0.6
lympho-cytes	11.5± ±1.1	8.1± ±0.5	10.6± ±0.9	10.2± ±0.6	4.2± ±0.6	4.1± ±0.2	4.6± ±0.2	10.6± ±1.0	7.9± ±0.5
neutro-phils	5.2± ±0.7	3.6± ±0.4	3.5± ±0.4	3.4± ±0.3	1.5± ±0.1	1.8± ±0.2	2.1± ±0.1	4.8± ±0.5	3.2± ±0.3
eosino-phils	0.78± ±0.06	0.33± ±0.02	0.22± ±0.04	0.28± ±0.03	0.26± ±0.02	0.35± ±0.02	0.30± ±0.03	0.46± ±0.06	0.35± ±0.02
Weight of the thymus, mg	32.0± ±1.0	31.6± ±1.3	24.1± ±1.8	26.0± ±1.7	25.8± ±2.6	32.0± ±2.1	27.4± ±0.9	33.4± ±1.2	29.0± ±0.8
Weight of spleen, mg	76.1± ±1.6	72.7± ±2.2	70.9± ±4.6	74.4± ±3.4	75.1± ±5.2	73.2± ±2.4	72.8± ±2.6	77.5± ±3.6	74.1± ±2.4
Bone marrow									
No. of karyocytes in thigh bone, mil.	23.7± ±1.4	24.1± ±2.2	22.6± ±1.4	24.2± ±1.4	24.8± ±1.3	25.2± ±1.2	22.4± ±2.1	22.4± ±1.2	23.7± ±1.1
mitotic index, %	6.3± ±0.3	4.1± ±0.3	3.0± ±0.2	3.5± ±0.3	4.5± ±0.4	4.0± ±0.3	4.9± ±0.6	5.5± ±0.3	4.5± ±0.2
no. of fissionable cells in thigh bone, thous.	150.0± ±6.2	98.8± ±5.8	67.8± ±4.6	87.0± ±5.0	110.8± ±6.1	100.8± ±5.2	108.0± ±6.4	123.2± ±5.6	104.4± ±4.2
KOE content in marrow of thigh bone (x 100)	29.65± ±1.5	27.85± ±2.26	29.69± ±0.57	40.51± ±2.4	31.02± ±1.52	21.25± ±0.82	25.26± ±1.78	30.84± ±1.06	30.39± ±0.7

TABLE 36

/137

DAILY CHANGES IN SEVERAL INDICES OF THE STATE OF THE HEMATOPOIETIC SYSTEM IN C57BL/6 MICE

Tested indices	Time during day of study, hr.								Average daily level
	05	08	11	14	17	20	23	02	
Formal elements of peripheral blood, thous. per 1 mm^3:									
leukocytes	25.9± ±2.8	26.1± ±1.7	22.2± ±1.4	16.2± ±1.3	10.2± ±1.2	10.6± ±0.9	14.2± ±1.3	17.0± ±1.1	16.5± ±0.8
lympho-cytes	18.6± ±1.2	19.1± ±1.4	16.3± ±1.0	11.3± ±1.1	7.3± ±0.8	7.4± ±0.7	9.4± ±0.8	11.5± ±0.7	12.6± ±0.5
neutro-phils	6.3± ±0.5	4.1± ±0.5	3.7± ±0.4	2.8± ±0.4	1.9± ±0.2	2.4± ±0.2	3.6± ±0.5	3.9± ±0.4	3.6± ±0.4
eosino-phils	0.56± ±0.09	0.64± ±0.07	0.58± ±0.12	0.42± ±0.08	0.14± ±0.04	0.16± ±0.03	0.22± ±0.02	0.38± ±0.07	0.38± ±0.02
Weight of thymus, mg	48.5± ±5.8	50.1± ±3.7	56.4± ±4.7	57.0± ±3.6	54.9± ±5.5	50.8± ±5.7	48.0± ±4.3	54.1± ±5.2	51.2± ±3.4
Weight of spleen, mg	72.5± ±2.8	73.8± ±2.6	72.8± ±3.5	64.3± ±3.6	76.8± ±4.1	78.0± ±4.0	67.7± ±2.5	68.9± ±2.5	71.8± ±1.2
Bone marrow: no. of karyocytes in thigh bone, mil.	29.9± ±1.9	29.6± ±1.7	29.2± ±1.0	30.2± ±0.7	32.5± ±1.7	34.7± ±2.4	32.9± ±0.8	33.4± ±1.1	31.7± ±0.7
mitotic index, ‰	8.3± ±0.8	5.3± ±0.4	4.6± ±0.6	4.9± ±0.5	5.3± ±0.4	6.4± ±0.3	6.5± ±0.3	5.8± ±0.5	5.9± ±0.3
No. if fissionable cells in thigh bone, thous.	244.1 ±15.2	156.2 ±6.8	134.0 ±6.0	148.0 ±3.5	172.5 ±7.2	221.0 ±7.2	213.0 ±2.4	196.0 ±6.0	185.6 ±3.4
KOE content in marrow of thigh bone (x100)	27.1± ±1.27	25.6± ±2.2	34.6± ±2.1	35.89 ±2.88	36.5± ±2.22	28.3± ±2.75	29.0± ±2.5	31.2± ±1.33	31.04 ±1.01

In analyzing the dependence of the formation of endogenic splenetic colonies on dosage it was found that D_0 remains more or less constant, regardless of the time of radiation during the day [93, 94].

In studying intact bone marrow in H mice by the method of exogenic splenetic colonies, an increase was found in the production of exogenic KOE 1½-2 times with the administration of 5×10^4 cells of bone marrow or 10^6 spleen cells taken from donors in the light period in comparison with the KOE production when cells were taken in the dark period [94]. Similar results were obtained in studying the KOE content in intact bone marrow of CBA and C57BL/6 mice (see Fig. 25, b and 26, b). Daily variations in the KOE content of bone marrow of intact mice are analogous in character to the curve reflecting the dependence of the formation of endogenic KOE on the time of radiation. On the basis of the above, it can be stated that change in the production of endogenic KOE, as the variation in survival of the animals, with radiation in different times of the day is determined, first of all, by variations in the initial content of hematopoietic cells in the body.

Another periodic process determining to some degree the existence of daily rhythms of radiosensitivity of the body are fluctuations in the number of fissionable hematopoietic cells (see Fig. 25, b, 26, b; Tables 35 and 36). The proliferative pool of bone marrow cells of CBA and C57BL/6 mice doubles by the start of the light period in comparison with its middle [44]. Periodic production of cells of bone marrow makes its contribution to periodic changes in the content of individual cellular elements in peripheral blood (see Fig. 25, b, 26, b; Tables 23, 33 and 34). Noteworthy is the fact that periods of increase in the pool of KOE of bone marrow in CBA and C57BL/6 mice coincide in time with a decrease in the pool of fissionable cells. Further studies are necessary to determine the rigidity of the connection between these two periodic processes.

As the course of the daily curve of radiosensitivity of CBA mice according to the criterion of life span in cases of ionizing radiation in

absolutely lethal doses is a mirror reflection of daily changes in the mitotic activity of bone marrow cells (See Fig. 27), it can be assumed that in this case longer or shorter life span depends primarily on the degree of radiation damage to fissionable, more-differentiated cells of bone marrow. Analogous data are given by Pizzarello et al. [78]. /139

The existence of daily rhythms of radiosensitivity in animals gives basis for assuming such in man as well. Experimental determination of the range of daily variations in the sensitivity of the human body to ionizing radiation will make it possible to introduce corresponding corrections in permissible doses for cosmonauts during long space flights.

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The Temporal Organization of Processes of Cell Reproduction and its Connection with Rhythms of Radiosensitivity of the Body

It is known that the biological effect of ionizing radiation on an animal or plant organism is largely connected with changes occurring after radiation in cell division processes. In recent years it has been revealed that radiation has different effects on cell division. These effects are due, on one hand, to the general suppression by radiation of processes necessary for entry of cells into the mitotic cycle, and on the other — to the development of blocks of cell transition from one phase of the cycle to another.

To characterize the cellular kinetics of the body, it is necessary to know the regularities in the temporal movement of cells through the mitotic cycle. A great deal of material has been accumulated in the literature indicating the existence of a daily periodicity in many processes of the mitotic cycle. Data have also been obtained concerning daily changes in the radiosensitivity of the body. If it is assumed that the biological effect of radiation on the body is connected with changes in cellular kinetics of its tissues, then we can assume the existence of a dependence between daily rhythms

of radiosensitivity and cell division in the body. Analysis of this question is of great interest both from the point of view of explaining mechanisms underlying rhythms of radiosensitivity and from that of working out protective measures against radiation under ground and space conditions.

The temporal organization of cell reproduction in the body. One of the central aims of modern chronobiology is to study the temporal organization of biological systems of various levels of complexity. According to Goodwin [59], the temporal organization of a system is formed by the spectrum of rhythmic processes with different periods, but correlated with each other in time. The concept of "temporal organization" is applied not only to morphologically formed and independent systems, for example, a cell, an organ or the organism as a whole, but also to systems of physiological processes performing a certain biological function. The complex of processes underlying cell reproduction can be considered such a functional system.

By temporal organization of cell reproduction must be understood the combination of rhythms (coordinated in time) of physiological processes occurring in the cell during its preparation for division and in division itself [41, 43].

The possibility of studying the temporal organization of cell reproduction /140 has become reality since the basic processes occurring in different phases of the mitotic cycle have been determined.

In analyzing the temporal organization of cell reproduction it is first of all necessary to know how the rhythmic increase in the mitotic index is expressed. In a number of works [61, 73], it has been shown that the rise in mitotic activity during the day is connected with the increase during that time of the duration of mitosis. However, a more detailed study of this matter has shown that although daily changes in the duration of mitosis are observed in cellular populations (Fig. 29, a) the rhythmic increase in the mitotic index is due to the increased number of cells entering mitosis per time

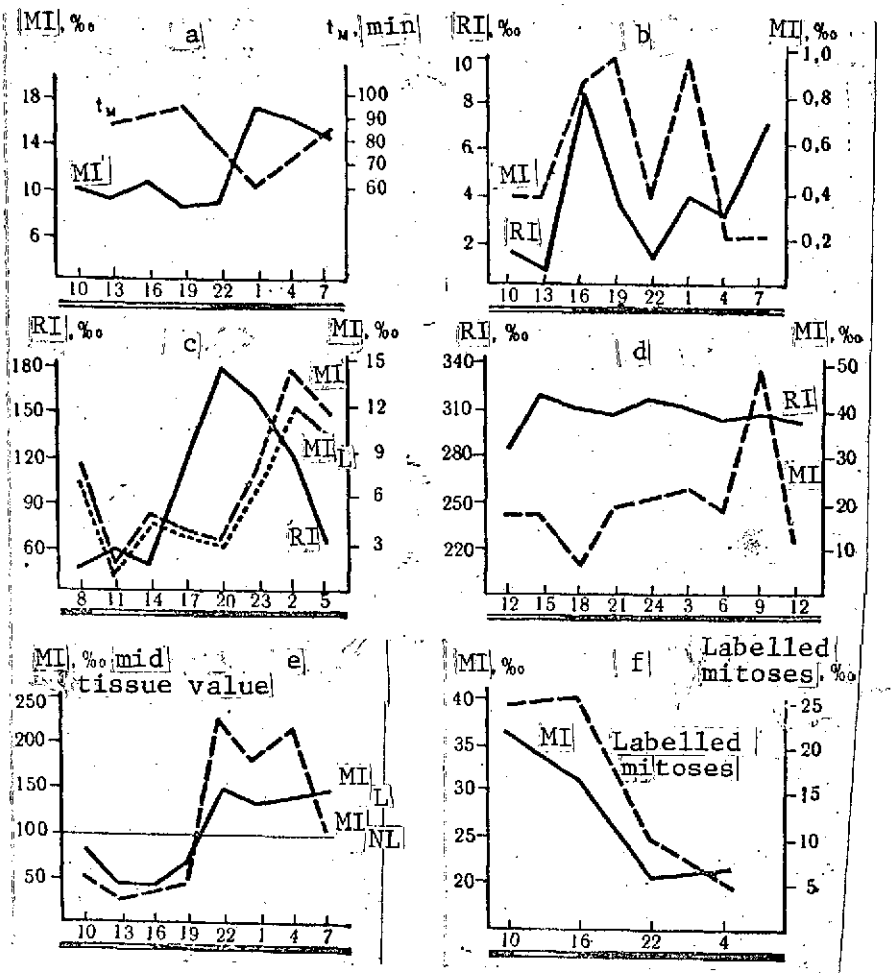

Figure 29. Temporal characteristics of several periodic processes.

a — diurnal rhythms of mitoses and duration of mitosis in basal epithelium of the esophagus of rats; b — diurnal rhythms of DNA synthesis and mitosis in the epithelium of the thyroid gland of mice; c — daily rhythms of DNA synthesis, mitoses and labelled mitoses in basal epithelium of the esophagus of mice; d — changes throughout the day in DNA-synthesizing and fissionable cells in the hypotetraploid strain of Ehrlich's ascitic tumor in mice; e — diurnal changes in indices of labelled and non-labelled mitoses in basal epithelium of mice; f — diurnal rhythms of mitoses and percentage of labelled mitoses in the epithelium of crypts of the small intestine of rats. MI — mitotic index; RI — index of DNA-synthesizing cells; t_M — duration of mitosis; MI_L — index of labelled mitoses, MI_{NL} — index of non-labelled mitoses

unit, i.e. synchronization of the movement of cells in the mitotic cycle [39, 41]. /141

In connection with the data obtained, the question has arisen of what changes in the mitotic cycle cause a rhythmic increase of fissionable cells during the day. In recent years a large number of works has been carried out indicating that the number of DNA-synthesizing cells in various tissues is subject to the regularities of a diurnal rhythm [43]. As DNA-synthesis serves as a necessary condition for the onset of mitosis in the diploid cell and a quantitative connection exists between these processes, on this basis a number of authors have expressed the opinion that the diurnal rhythm of fissionable cells is completely determined by diurnal rhythmic variations in the number of cells passing through the S-phase of the mitotic cycle [33, 53]. From this point of view the synchronizing mechanism responsible for the periodic increase in the fraction of cells in the mitotic cycle and synthesizing DNA must be considered the only generator of rhythms of mitoses. A number of observations confirms the existence of such a mechanism. For example, in the epithelium of the thyroid gland of mice a two-phase diurnal rhythm of DNA-synthesizing cells is found; the diurnal rhythm of mitoses has an analagous character (Fig. 20, b) [39].

A similar picture, according to our data, is often recorded in the epithelium of crypts of the small intestine of rats (see Fig. 30). The results of the following experiments favor the connection between the synchronization mechanism of the entry of cells into the S-phase of the mitotic cycle and the diurnal rhythm of mitoses [38]. With the administration of thymidine-H^3 to mice 5, 6 or 8 hours before examination of mitotic activity, during the rise in its rhythm the major portion of fissionable cells is labelled, i.e. they have passed through the S-phase of the mitotic cycle before mitosis. For example, in the basal epithelial layer of the esophagus the number of cells labelled with the isotope at that time reached 73-77% (Fig. 29, c).

It can, therefore, be concluded that the mechanism synchronizing the cellular population before the synthetic phase of the cycle at a certain time

of the day takes part in the formation of diurnal changes in mitotic activity. It has been related [39, 43] to one of the intracyclic mechanisms of the diurnal rhythm of mitoses forming the temporal organization of cell reproduction.

Usually the rise in mitotic activity in the diurnal rhythm is detected, depending on the tissue being tested, in 1-5 hours after the increase in the number of DNA-synthesizing cells. We compare the temporal shift in phases of these rhythms in general with the duration of Stage G_2 of the mitotic cycle. However, both according to literature data and our own material, this temporal mutual relation between phases of diurnal rhythms of DNA synthesis and cell division does not always occur. The phase shift can be much greater and cases have been noted of active phases of rhythms occurring at the same hours of the day. What is more, there is an observation of increased mitotic activity without the preliminary increase in the number of DNA-synthesizing cells. For example, in the hypotetraploid strain of Ehrlich's ascitic tumor in mice on the 5th day of its development, with distinct diurnal variations in mitotic activity, no diurnal changes are seen in the amount of DNA-synthesizing cells (Fig. 29, d).

Determination of parameters of the mitotic cycle in the population of these cells showed that the time of their G_2-phase is found within wide limits, in some cells reaching 9-10 hours or even a day. These data can be explained /142 if it is assumed that cells synthesizing DNA are subject to still another synchronization in their later movement through the cycle before mitosis in the G_2-phase. As is known, Gelfant [58] isolated the so-called G_2-population in proliferating cellular systems; this consists of cells leaving the mitotic cycle at a certain time in the G_2-phase and then able to enter mitosis. In a number of experiments [42, 43] we obtained proofs of the existence of the G_2-population of cells in various tissues. For example, in basal epithelium of the esophagus of mice, even 6 hours after the administration to the animals of thymidine-H^3, there is a significant (to 27%) proportion of labelled mitoses which can indicate division of cells in the G_2-population. In parenchymatous cells of the liver at isolated hours of the day, the number of unlabelled mitoses in the same test can reach 23%. However, the main thing is that the

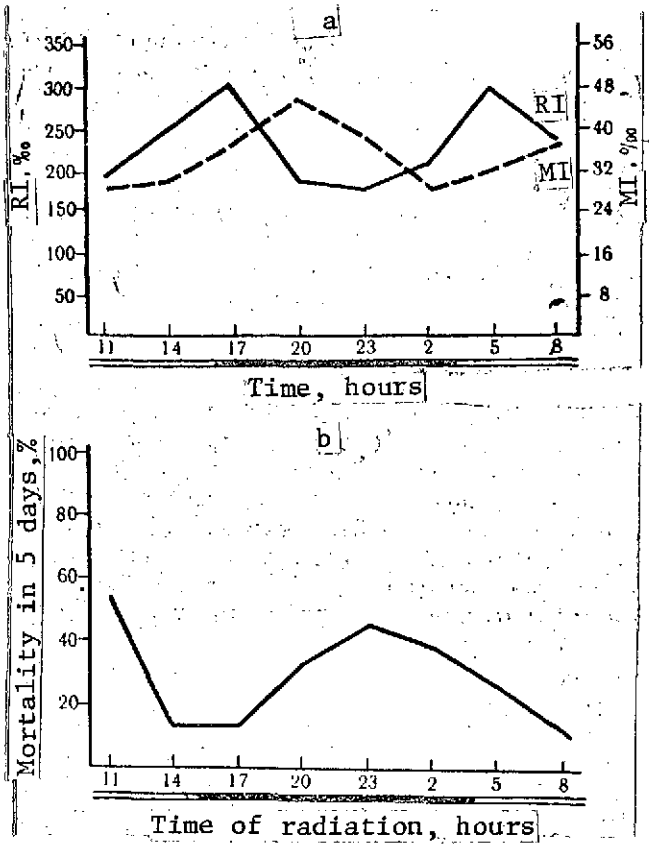

Figure 30. Diurnal rhythms of mitoses (MI) and DNA-synthesizing cells (RI) in the epithelium of crypts of the small intestine of Wistar rats (a); diurnal rhythm of radiosensitivity of Wistar rats (b).

index of unlabelled mitoses changes rhythmically throughout the day; the amplitude of fluctuations during the day in the index of unlabelled mitoses was almost 3 times greater than that of labelled mitoses. The index of unlabelled mitoses during the period of increased rhythmic mitotic activity increases more sharply than that of labelled mitoses (Fig. 29, e).

These data served as the basis for concluding the existence of a second intracyclical mechanism of the diurnal rhythm of mitoses, synchronizing the kinetics of cells in the G_2-phase before mitosis and forming the temporal /143
organization of cell reproduction [39]. As seen from Fig. 29, e, the rhythmic increase in the number of labelled fissionable cells occurs at the same time of day when a rise is also observed in the number of labelled mitoses. This fact, evidently, indicates that the effect of the synchronizing mechanism in the G_2-phase applies both to "transit" cells and to those detained in this

phase which are found before mitosis. One more argument in favor of the existence of a mechanism synchronizing the entry of cells into mitosis from the G_2-phase of the cycle is the formation of a daily rhythm of the number of labelled mitoses in the epithelium of crypts of the small intestine in rats (Fig. 29, f). Changes in the percent of labelled mitoses throughout the day with the same constant interval of fixation of material after the administration to the animal of thymidine-H^3 (1 hour) indicate different (in different hours of the day) duration of the G_2-phase in cells of the crypts of the small intestine.

A number of authors [85] reported diurnal fluctuations in the number of silver grains over the nucleus of cells labelled with thymidine-H^3. Analogous data have also been obtained in our own studies of various cellular populations [39, 43]. Other conditions being equal, the number of silver grains over the nucleus of the cells can to a certain degree reflect the intensity of DNA synthesis. Therefore, rhythmical changes in this index indicate the possibility of diurnal changes in the rate of DNA synthesis in cells and correspondingly in the length of the S-phase of the mitotic cycle.

At the present time, therefore, it is known that the cellular population throughout nearly the entire mitotic cycle is characterized by diurnal rhythmic changes. Diurnal rhythms are found in the number of cells entering the S-phase of the cycle, in the rate of cells passing through the S-phase, in the length of the G_2-phase of the cycle, in the number of fissionable cells and, finally, in the length of cell division itself.

While we do not have available specific data on the state of cells throughout the day in the G_1-phase of the cycle, existing materials infer that diurnal periodicity is also typical of these cells. Coincidence in time of these rhythms and, evidently, also those of other physiological processes involved in cell division is ultimately expressed by the temporal organization of cell reproduction.

The most important paths of regulating and preserving the temporal organization of cell reproduction with which the state of homeostasis and the dynamic continuance of the cellular system are closely connected are, in our opinion, the above-characterized intracyclic mechanisms of diurnal rhythms in the kinetics of cells. If, as already noted earlier, the complex of processes of the mitotic cycle are considered as a single functional system, then these mechanisms of the temporal organization of cell reproduction must be considered as intrasystemic. However, the organization of cell multiplication in time is not independent of periodic changes in other functions of cells, in the body and, finally, in the environment surrounding the animal or plant. In connection with this, besides intrasystemic mechanisms of the temporal organization of cell regulation, there are also extrasystemic mechanisms through which cell division in the organism is regulated and proliferative processes are affected by the environment. The function of these and other mechanisms must be definitely connected with each other in time, as otherwise phase error will occur, which will inevitably lead to disturbances in the temporal organization of cell division. /144

It is known that many hormones have a strong effect on cell division processes. However, study of the action of thyroxine on cellular proliferation in animals has shown that the hormonal effect is variable throughout the day and depends on the phase of the rhythm of the cellular population, passing through a certain stage of the mitotic cycle [38, 39, 41, 42, 43].

In connection with this there is the opinion that diurnal rhythms exist in the sensitivity of cells to factors affecting their reproductive processes [30]. Theoretically, at any moment of the day cells can be found which are in each stage of the mitotic cycle, but their reaction to hormones appears only when at that time they are characterized by a certain phase of the diurnal rhythm of processes of some stage of the cycle. It was also found that the effect of thyroxine on cell division depends on the phase when the hormone was administered to the animals: in the phase of its natural increase in the blood in the diurnal rhythm or in the phase when its concentration is reduced. This indi-

cates a coordination in time of the rhythms of cellular kinetics with rhythms of body systems (the endocrine system) affecting cellular proliferation.

In a number of other experiments we found that photoperiodicity and illumination regimes are of essential significance in forming phase dynamics of daily rhythms of cell reproduction. At the same time, levelling the rhythm of locomotor activity in animals caused practically no phase shifts in diurnal rhythms of DNA-synthesizing and fissionable cells. Thus, photoperiodicity concerns a number of basic intrasystemic time clocks affecting the rhythms of cellular proliferation. Placing animals at this or any time in small boxes led to phase shifts in the rhythms of cell division. In all these variants of experiments, phase characteristics of the rhythm of cell reproduction changed most in a slowly-renewing cellular system (liver) and comparatively little in a rapidly renewing tissue (epithelium of the small intestine).

The studied regularities in temporal organization of cell reproduction are diagrammed in Fig. 31.

The existence in cellular systems of rhythms of sensitivity to factors altering cell function, including those which ensure their reproduction, are, in our opinion, of great interest in studying the reaction of cells to ionizing radiation. It can be assumed that in this case also there is a close connection between the periodically changing state of cells in a particular phase of the mitotic cycle and the biological effect of radiation. In other words, diurnal fluctuations in radiosensitivity of any cellular population and, evidently, the body as a whole are due to the fact that radiation acts on an oscillating system which at each moment of time is in a different phase state; radio damage must increase sharply if this state is most favorable for the development of physico-chemical, morphological and functional reactions caused by ionizing radiation.

Diurnal rhythms of the radiosensitivity of the body of animals with intestinal radiation injury. From the moment the first report on the existence

/145

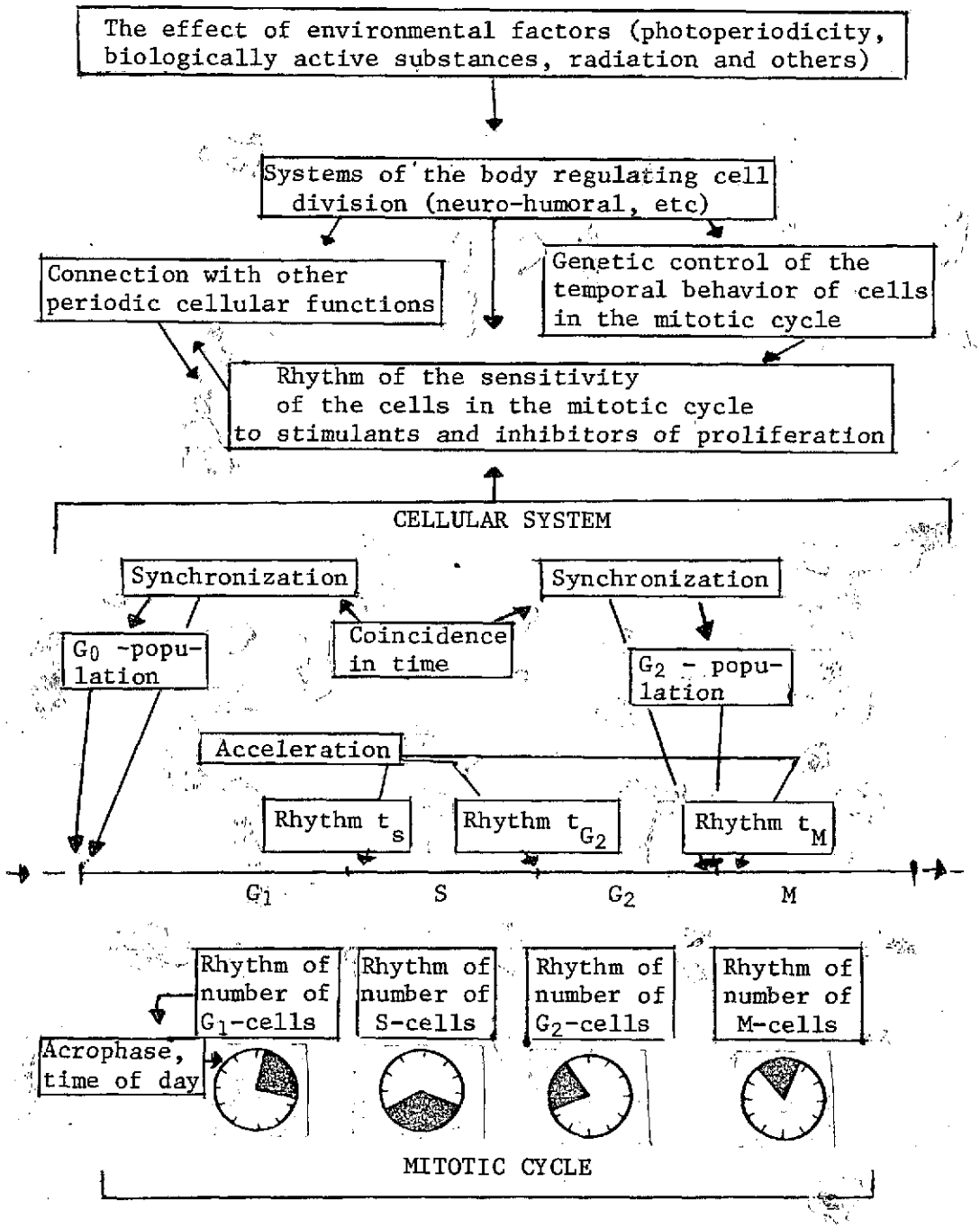
The effect of environmental factors (photoperiodicity, biologically active substances, radiation and others)
Systems of the body regulating cell division (neuro-humoral, etc)
Connection with other periodic cellular functions
Genetic control of the temporal behavior of cells in the mitotic cycle
Rhythm of the sensitivity of the cells in the mitotic cycle to stimulants and inhibitors of proliferation
CELLULAR SYSTEM
Synchronization
Synchronization
G_0 -population
Coincidence in time
G_2 - population
Acceleration
Rhythm t_s
Rhythm t_{G_2}
Rhythm t_M
G_1
S
G_2
M
Rhythm of number of G_1-cells
Rhythm of number of S-cells
Rhythm of number of G_2-cells
Rhythm of number of M-cells
Acrophase, time of day
MITOTIC CYCLE

Figure 31. Diagram of the temporal organization of cell reproduction in the body.

of daily rhythms in the sensitivity of the body to the effect of ionizing radiation appeared [Pizzarello, 79], the attention of the majority of researchers has been turned to revealing the dynamics of diurnal variations in radiosensitivity with radiation in the range of doses leading to so-called hematopoietic death of animals. Attempts have been made to explain the existence of rhythms in radiosensitivity by daily fluctuations in the pool of stem and fissionable cells. /146

In the literature we have found no works dealing with daily rhythms in the radiosensitivity of animals with radiation in the range of doses leading to primary damage of the epithelium of the gastro-intestinal tract.

Studies in this direction are important, not only for finding the limits of fluctuations in the radioresistance of various animals under natural conditions, but also for determining the connection between the rhythmic movement of proliferating cells of intestinal epithelium through the mitotic cycle and their radiosensitivity. Our own experimental studies were conducted on Wistar rats kept in groups with a regime of 12 hours of light and 12 hours of darkness. The animals were fed at the start of the active (dark) period of the day. Fig. 30, b, gives data on mortality of the animals in different groups during the first 5 days after a radiation dose of 850 R (γ-rays of Cs^{137}, dose rate — 47 R/min.) at different times of day. As with primary damage to the hematopoietic system, the degree of the gastro-intestinal syndrome varies, depending on the time radiation is applied during the day. The radiosensitivity of animals increases in the middle of the light (11 hours) and dark (20-2 hours) periods ($P < 0.05$).

As the gastro-intestinal syndrome and death caused by it reflect synergism of effects resulting from damage to a number of tissues, primarily the epithelium of the gastro-intestinal tract and the myelocyte renewal system in bone marrow [50], then the question arises of whether the existence of diurnal rhythms in radiosensitivity in cases of the gastro-intestinal form of radiation injury is due, as in the case of the hematopoietic form, to changes

in the pools of stem and fissionable hematopoietic cells. However, with radiation of random-bred rats at different times of the day in doses leading to primary damage of the hemopoietic system, increases in radiosensitivity usually coincide with changes in the light regime: from light to dark and from dark to light [6], i.e., they appear at a different time of day than in cases of the gastro-intestinal form of death. Interlinear differences in the course of the diurnal curve of radiosensitivity, at least in mice, are unimportant. Therefore, it can be assumed that the increased probability of "intestinal" death in case of radiation in the middle of the light and dark periods is due to diurnal variations in the state of the pool of stem and proliferating cells of intestinal epithelium. It is assumed that stem cells of intestinal epithelium are found in crypts along with the pool of fissionable and maturing cells; however, they are morphologically indistinguishable.

It is impossible to determine the daily changes in the number of stem cells, as well as their individual radiobiological parameters (n, D_0) because of the lack of a method of transplanting them. On the basis of comparison of diurnal variations in the pool of stem and fissionable cells in the hematopoietic system it can be assumed that at the moment mitotic activity is increased in epithelial cells of the intestine (17-20-23 and 8 hours)(see Fig. 30, a), as also in bone marrow, the content of stem cells is reduced. /147

As seen from Fig. 30, a, b, increases in radiosensitivity of the body with the gastro-intestinal form of death in individual periods of the day coincide almost completely in time with rises in mitotic activity in epithelial cells of crypts and with the start of increased DNA synthesis. Some temporal differences in the dynamics of these rhythms are, evidently, due to specifics of processes comprising them.

Radiosensitivity of individual phases of the mitotic cycle was studied in detail in synchronous cell cultures and in several biological objects. It was found that radiosensitivity changed essentially according to phases of the mitotic cycle, depending on the kind of cells, evaluation criteria and the radiation dose. As was found in tests on partially synchronized HeLa

cell populations, according to the criterion of survival, cells are most sensitive during mitosis, as well as in later G_1- or early DNA-synthesizing stages. With radiation in doses of 300 rad determination of the proportion of surviving cells showed that sensitivity directly before DNA synthesis is approximately 4 times higher than the later S-phase and during the major portion of G_1- and G_2-periods. Sensitivity of cells in mitosis is approximately 3 times higher than in late G_1- and early S-phases.

Thompson and Humphrey [89] in studies on a synchronous culture of L-P59 cells established that radiosensitivity of individual phases, according to the criterion of cell survival, increases from G_1 to S and G_2. Radiosensitivity of cells in phase G_2 increases as they approach mitosis. Fissionable cells are more radiosensitive than cells in G_2-phase. Shermann and Quastler, studying the fate of epithelial cells of the small intestine radiated at different periods of the mitotic cycle, established that the majority of disintegrating cells are found when radiation occurs in the S-phase. Bone marrow cells, according to the number of disintegrating elements and cells with chromosome aberrations,are more sensitive in the DNA-synthesis period and in the post-synthetic period in comparison with cells radiated in the G_2-period [17].

In mouse spermatocytes it was shown that there are inverse ratios between the effect of radiation, according to the criterion of cell death, and the appearance of chromosome breaks, although they assume that weakly damaged cells can be eliminated from the population before the appearance of chromosome injuries. In fibroblasts of L-P59 mice, according to the criterion of the development of chromosome damages, most sensitive is the S-phase; the G_1-period is half as sensitive; with radiation in the G_2-period there are slightly more aberrations than in G_1. According to the criterion of delay of mitosis, most sensitive are cells in G_2- and S-phases, least sensitive those in the G_1-period [54].

Sinclair [86, 87] feels that cells of mammals can be divided into two types according to the specifics of radiosensitivity: cells with a brief G_1 are most sensitive in mitosis and the G_2-phase, as well as in the G_1-phase and least sensitive during the latter part of the S-period; cells with a long G_1, in addition to the above, have a resistant stage at the start of G_1, after which follows a sensitive stage at the end of the G_1-period. The latter can be as sensitive as mitosis.

If our assumption is correct, that the daily rhythm of radiosensitivity according to development of "intestinal" death reflects more or less synchronous progress of epithelial cells of the small intestine throughout the day through the cycle, then most sensitve are cells at the end of the G_1- and the /148 start of the S-period, as well as fissionable cells. Further studies are necessary to define more accurately the above assumptions. Pizzarello and Witcofski [78], in cases of the hematopoietic form of radiation damage, established a coincidence in time of day between increased radiosensitivity of animals according to the criterion of their average life span and the rise of mitotic activity in bone marrow and an absence of such coincidence with increased DNA synthesis.

N75 14422

Development and Creation of Conditions for the Radiation of Biological Objects with High Energy Protons

In our experiments the proton beam emitted from the synchorcyclotron was widened by filters of alternating layers of polyethylene and lead. By changing the thickness of filters, proton dose fields of various energies could be created.

The thickness of the layers and their location in the filter providing the required proton energy and evenness of the dose field were determined by a method which we devised to calculate the distribution of protons beyond the

filter, taking into account changes in their energy. Using the concept of the distribution function [83, 56], it could be shown that the distribution of particles f(t, r) in a plane perpendicular to the direction of the beam and S distance away from the filter is characterized by the Gauss principle:

$$f(t, r)\, d\sigma = \frac{1}{\pi \bar{r}^2} \exp\left(-\frac{r^2}{\bar{r}^2}\right) d\sigma,$$

where dσ is element of area at distance r from the center of the beam; $\bar{r}^2 = 4(A_2 + 2sA_1 + s^2A_0)$ is the standard deviation of the protons; A_0, A_1 and A_2 are coefficients depending in a complex way on the multiple proton scattering angle and thickness of the filter:

$$A_0 = \frac{\alpha}{4}\left[at + b \ln \frac{R_0}{R_0 - t} + \frac{ct}{R_0 (R_0 - t)}\right];$$

$$A_1 = \frac{\alpha}{4\rho}\left\{(t - R_0)\frac{A_0}{\alpha} + \frac{a}{2}\left[R_0^2 - (R_0 - t)^2\right] + bt + c \ln \frac{R_0}{R_0 - t}\right\}$$

$$A_2 = \frac{\alpha}{4\rho^2}\left\{-(R_0 - t)^2 \frac{4A_0}{\alpha} - \frac{8\rho}{\alpha}(R_0 - t) A_1 + \frac{Q}{3}\left[R_0^3 - (R_0 - t)^3\right] + \right.$$

$$\left. + \frac{b}{2}\left[R_0^2 - (R_0 - t)^2\right] + ct\right\},$$

where ρ is the density of the filter material:

$$\alpha = \frac{0{,}628 \cdot z^2 K_N}{N_A} \ln\left(181 \cdot z^{-1/3}\right),$$

$K_N = \frac{N_A}{M}$ is the number of scattering nuclei per 1 g substance with a molecular weight of M; z is the scattering nuclear charge; N_A - Avogardo number; a, b and c are coefficients whose values for the energy range between 100 and 660 Mev are given in Table 37.

TABLE 37

VALUES OF COEFFICIENTS a, b AND c FOR DIFFERENT MATERIALS

Material	Coefficient		
	a	b	c
Polyethylene	$-1.54 \cdot 10^{-7}$	$1.78 \cdot 10^{-4}$	$1.63 \cdot 10^{-4}$
Carbon	$-1.85 \cdot 10^{-7}$	$2.15 \cdot 10^{-4}$	$1.94 \cdot 10^{-4}$
Aluminum	$-2.07 \cdot 10^{-7}$	$2.5 \cdot 10^{-4}$	$2.25 \cdot 10^{-4}$
Iron	$-2.0 \cdot 10^{-7}$	$2.75 \cdot 10^{-4}$	$3.7 \cdot 10^{-4}$
Lead	$-1.47 \cdot 10^{-7}$	$3.6 \cdot 10^{-4}$	$2.12 \cdot 10^{-3}$

Results of calculation of r^2 for various filters with $S = (550 - \frac{t}{\rho})$ are given in Table 38.

TABLE 38

RESULTS OF CALCULATION OF $\overline{r^2}$ FOR VARIOUS FILTERS

Configuration of filter	Energy of protons beyond the filter, Mev	$\overline{r^2}$, cm^2
10 cm Pb	510	2856
85 cm $(CH_2)_n$ + 5 cm Pb + 28.4 cm $(CH_2)_n$	240	2364
5 cm Pb + 142 cm $(CH_2)_n$	~120	1902
85 cm $(CH_2)_n$ + 5 cm Pb + 56.8 cm $(CH_2)_n$	~120	2659
142 cm $(CH_2)_n$ + 5 cm Pb	~120	6257

According to these data, dose rates R of protons beyond each filter were evaluated using the expression:

$$P = \frac{\Pi \cdot 1{,}6 \cdot 10^{-8} \frac{dE}{dx}}{\pi \overline{r^2}},$$

where Π is proton flux per unit of time entering the filter; $\frac{dE}{dx}$ are ionization losses of protons leaving the filter.

Table 38 shows possible change in the configuration of filters which can provide the same proton energy.

This method was used to select the following configurations for filters to create proton dose fields of various energies:

10 cm Pb (E = 510 Mev)

85 cm $(CH_2)_n$ + 5 cm Pb + 28.4 cm $(CH_2)_n$ (E = 240 Mev)

85 cm $(CH_2)_n$ + 5 cm Pb + 56.8 cm $(CH_2)_n$ (E = 120 Mev)

One disadvantage of the method of forming proton dose fields with the use of filters is the presence in the dose field of secondary radiation (basically cascade protons and neutrons) generated by nuclear interactions between protons and filter material. In connection with this, a method was devised for calculating the discharge of secondary nucleons from different filters, taking into account attenuation and multiplication in the filter of primary protons and secondary nucleons. Calculations show that the discharge of secondary nucleons increases with increased thickness of the filter, reaches maximum with thicknesses about 60 g/cm^2 of polyethylene and 100 g/cm^2 of lead and is then reduced as the result of inhibition and absorption of nucleons in the filter. /150

For the selected filter configurations providing energy of 510, 240 and 120 Mev, the contribution of secondary radiation to the complete absorbed dose, evaluated with the use of this method, was 1, 2.2 and 1.8% respectively.

A proton dose field with energy under 100 Mev could theoretically be formed in an accelerator with proton energy of 660 Mev using filters as indicated above. However, calculations show that because of the straggling of ionization losses proton monochromatism will be strongly disturbed; therefore, a proton dose field with energy of 50 Mev was formed by inhibiting the first proton beam in the filter (183 cm polyethylene) and deflecting the hindered beam with a magnetic field into one of the collimators. As subsequent measurements showed, this improved the monochromatism of the proton beam and reduced the contribution of attendant secondary radiation.

A complex of dosimetric and electron-physics equipment was created for experimental study of the basic characteristics of dose fields (evenness of the radiation field, tissue dose and proton energy), necessary in radiobiological experiments, and for controlling the radiation of biological objects in a synchrocyclotron.

The evenness of the proton dose field was measured using ILK dosimeters, ferrosulfate chemical dosimeters and thermoluminescent glass dosimeters [1, 37]. Besides these dosimeters, tissue-equivalent ionization chambers of the condenser type were used. All dosimeters were calibrated with a standard Co^{60} source and in proton fluxes of various energy.

To determine proton flux carbon [C_{12} reaction $(p, rps_{11}]$ and aluminum [reaction of $Al^{27}(p,3pn)Na^{24}$] activated detectors were used. These detectors were calibrated by photoemulsions and a Faraday cylinder, absolute methods of measuring particle flux. It was found that total error of the dosimeters does not exceed 10%. These dosimeters were used together with activating carbon detectors to measure the evenness of a proton dose field with average energy of 50 Mev, which was not greater than 7%.

The "rigid escapement" and high threshold of registration of protons (about 20 Mev in condenser chambers and ILK), determined by the thickness of the dosimeter wall, prevent the use of these dosimeters when the presence of low-energy and strongly ionized radiation can be expected in the dose field. Therefore, in order to measure the cartogram of proton dose fields (with energy of 510, 240 and 120 Mev), a special apparatus was designed consisting of a thin-wall tissue-equivalent isotrope ionization chamber and mechanical construction making it possible to rotate the chamber around the center of /151
the dose field. The front hemisphere of the outer electrode was made of copper screen 0.5 mm thick and about 75% transparent. The chamber was filled with a tissue-equivalent gas mixture and sealed with a rubber coating about 50 μμ thick; because of this the threshold of proton registration was only 1.5-2 Mev. The current of the chamber during its movement around a radius

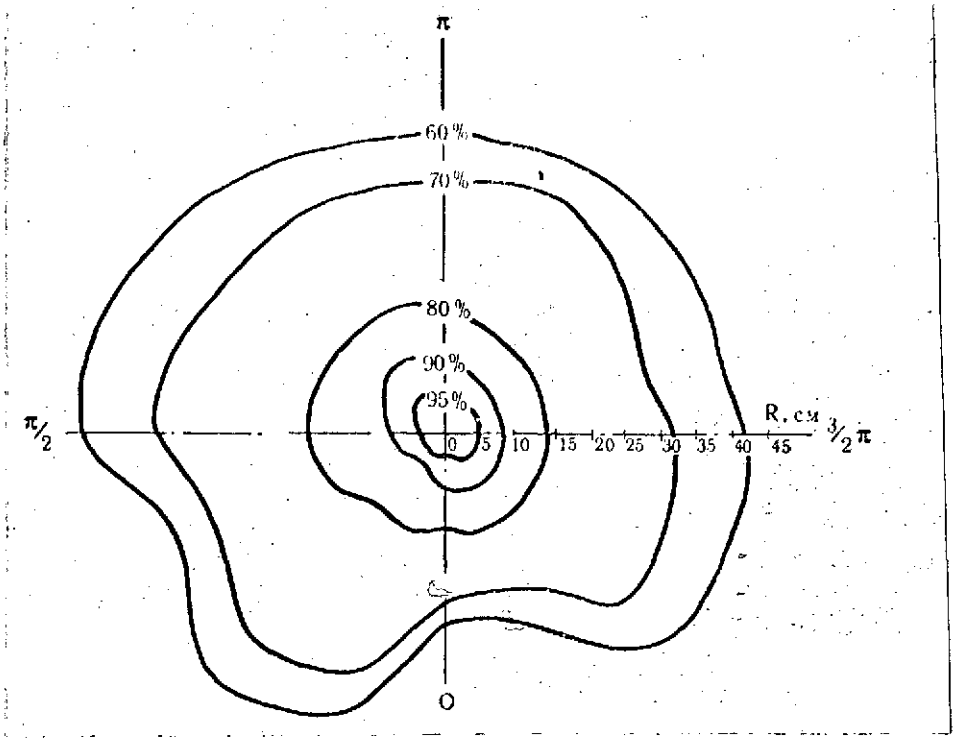

Figure 32. Cartogram of proton dose field with energy of 510 Mev.

of up to 50 cm was registered on recorder tape. Such data were used to determine dose distribution along the radius at various angles from 0 to 2 π, according to which a cartogram of the dose field was then plotted.

As an example Fig. 32 gives the cartogram of a proton dose field with energy of 510 Mev. Analogous measurements were made with proton energies of 240 and 120 Mev. The measurements established that doses calculated according to the above outlined method differ from measured values by not more than 10%; this maximum divergance is observed only at the edge of the dose field with a radius of 50 cm. These measurements also showed that layered filters can be used to create proton dose fields of various energies with ±20% evenness in a field up to 50 cm in diameter.

One of the basic characteristics of the dose field is the proton energy spectrum. For spectrometry in a wide energy range and identification of particles a method was devised based on amplitude analysis of impulses with maximum amplitude developing in the second detector of a telescope consisting of two semiconductor detectors and containing a filter of variable thickness [36].

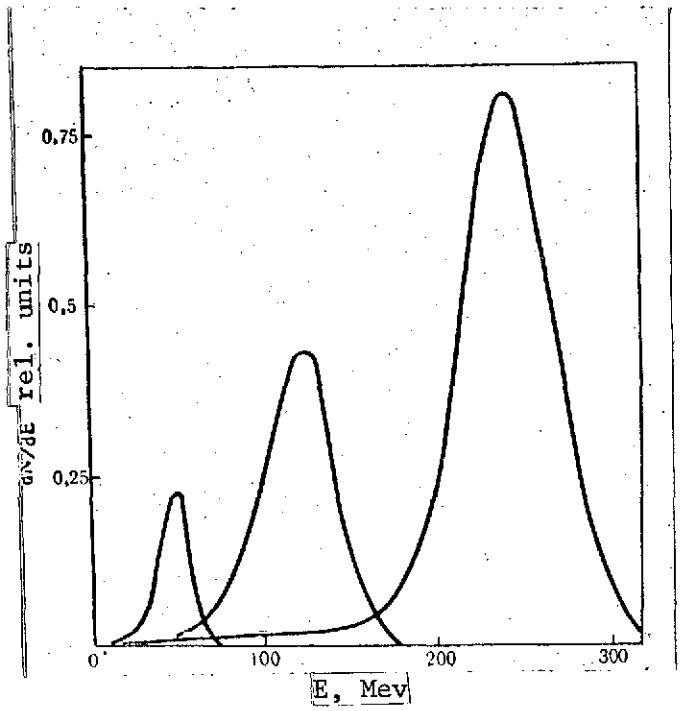

Figure 33. Measurements of proton energy spectra /152

Measurements of proton energy spectra with average energy of 50, 120 and 240 Mev [22] are given in Fig. 33. Particles with charges greater than one were not recorded. Measurements also showed that the contribution of low-energy secondary protons to the dose field is not great. This is due to the angular distribution of secondary radiation and the large distance between the dose field and the filter.

In conducting radiobiological experiments in which detailed knowledge of the energy spectrum was not required, the average energy of the proton beam was determined by the attenuation curve in plexiglass (to 90 Mev), aluminum (to 140 Mev) and steel (to 240 Mev). The attenuation curve was measured by a re-

mote control unit in which the thickness of the absorber in front of the ionization chamber was changed by moving a key. Changes in the thickness of the absorber and the corresponding measurement of ionization current were registered on recording tape.

Radiation of biological objects was controlled by a system of monitor chambers.

The ionization current created by protons in the monitor chambers was recorded by an electronic circuit converting the current to voltage pulses. In order to increase the accuracy of dosage, a specially developed unit was included in the control circuit of the synchrocyclotron to detach the accelerator when a given dose is reached in the experiment.

As it is customary to bring the biological effect into conformity with the absorbed dose (mid-tissue, surface dose, dose to a critical organ), in each radiobiological experiment it is necessary to have information on the spatial distribution of the absorbed dose in the radiated object both to calibrate the monitor chambers controlling radiation and to interpret the results of radiobiological experiments. With regard to small biological objects (microobjects, seeds, etc.), the absorbed dose reaching them is practically absent and the average tissue dose in this case is numerically equal to the exposure dose, expressed in units — tissue rad. /153

In the radiation of larger objects (small laboratory animals and dogs) it is necessary to study the spatial distribution of the absorbed dose.

It has been found that the dose absorbed in an irradiated object at depth t at a point whose coordinate is equal to residual range $R = R_0 - t$ of the proton can be expressed as

$$D(R) = \frac{15{,}3\sqrt{\pi}\sqrt{|R|}}{2\sqrt{2\sigma_t^2}}\left[I_{-1/4}\left(\frac{R^2}{4\sigma_t^2}\right) + (Sgn R)\, I_{1/4}\left(\frac{R^2}{4\sigma_t^2}\right)\right] + 0{,}7\left[1 + \Phi\left(\frac{R}{\sigma_t}\right)\right],$$

where $I_{-1/4}, I_{1/4}$ are modified Bessel functions; $\Phi\left(\frac{R}{\sigma_t}\right) = \frac{2}{\sqrt{2\pi}} \int_0^{\frac{R}{\sigma_t}} e^{-\frac{z^2}{2}}\, dz$ is the integral of probability; $\sigma_t^2 = \sigma_0^2 + \sigma_{R_0}^2 - \sigma_{R_0-t}^2$ is dispersion of the proton beam flux at depth t; σ_0^2 is the initial dispersion of the proton beam; $\sigma_{R_0}^2, \sigma_{R_0-t}^2$ is dispersion of the proton beam with ranges R_0 and $(R_0 - t)$ respectively.

The size of a proton radiation field with energy of 50 Mev only allowed radiation of small laboratory animals — mice and rats. The container consisted of sections in each of which one mouse was placed in order to avoid screening during radiation. The size of the sections made it possible for the mice to move freely; therefore, their radiation can be considered isotropic. To calculate depth distribution of the absorbed dose in a mouse phantom which it is reasonable to represent in the form of a ball with radius R, the distribution of the absorbed dose in the object was found by calculation using an experimental proton attenuation curve in tissue-equivalent material. In our work this curve was obtained for plexiglass with the help of a thin-wall ionization chamber filled with a tissue-equivalent gas mixture.

Dosage at a point r from the center of the ball was:

$$D(r) = (A - Bc^2) - B\left(R^2 - \frac{r^2}{3}\right) + BCR + \frac{BCR}{2r}(R^2 - r^2)\ln\frac{R+r}{R-r},$$

where A, B and C are constants in the analytic expression of the experimental attenuation curve $D(t) = A - B(t - C)^2$.

Calculations showed that the dose increases from the surface to the center of a phantom with radius R = 1.68 cm; however, the difference in the absorbed dose is only 11%. Mid-tissue dose was determined by integrating D(r) through the ball; the integral from the last term in D(r) was obtained by numerical integration: /154

$$D_{\text{mid·tis}} = \frac{3}{R^3}\int_0^R D(r)\, r^2 dr = A - BCR - 0{,}8BR^2 + 0{,}16BCR^4.$$

Study of the distribution of the ends of the proton range is of especially great importance as it is at the end of the range that maximum losses of energy are observed. Therefore, if radiation conditions are such that the ends of ranges are located in a particular organ of the animal, then the biological effect cannot be connected with mid-tissue dose. The distribution of the ends of the range is the particular case of the distribution of protons along residual ranges $\rho = R_0 - t$ when the residual range is zero. It is easy to show that this distribution is

$$N(r) = \frac{\pi}{r R_0^2} (R_0^2 + R^2 - r^2),$$

where R_0 is the range of protons hitting the phantom.

If protons are distributed along a certain energy spectrum $\frac{dN}{dE}$, then

$$N(r) = \int_{R_{0\,min}}^{R_{0\,max}} N(r) \frac{dN}{dR_0} dR_0,$$

where $\frac{dN}{dR_0} = \frac{dN}{d\varepsilon} \cdot \frac{d\varepsilon}{dR_0}$ is the spectrum of protons falling along the ranges.

Calculations have shown that the density of the ends of ranges for the proton energy spectrum with average energy of 50 Mev increases from the surface to the center. Maximum differential observed on the surface of a phantom with a radius R = 1.68 cm is 44% with mean deviation from average of 11%. We used these calculation methods in planning radiobiological experiments: justification of the criterion of mid-tissue dose, creation of physical conditions for uneven radiation, etc.

In radiating large laboratory animals, depth distribution of the absorbed dose was studied by both calculation and experimental methods. In an experimental study of the spatial distribution of the absorbed dose, we used a cylindrical plexiglass phantom, a homogeneous phantom reproducing the geometric form of a dog weighing 12 kg made of tissue-equivalent material and a heterogeneous bone-paraffin phantom. The basis of the heterogeneous phantom was the natural skeleton of a dog, muscle tissue was simulated by a mix-

ture of paraffin (81%) and silica filler (19%) and lung tissue by gelatine capsules.

The first two phantoms were used in analyzing calculation and experimental dosimetric methods, as well as in those radiobiological experiments in which, on the basis of calculations, even distribution of absorbed dose could be expected in the radiated object.

In experiments with uneven radiation of animals, as well as in cases when it is necessary to study the radiation load on individual parts of the body of an animal, phantom measurements were conducted on the heterogeneous phantom. /155

Tissue-equivalent condenser chambers were used to measure absorbed dose in the phantoms.

Thus, in a synchrocyclotron with unregulated energy of 660 Mev, proton dose fields of various energy up to 50 Mev were created and a complex of equipment created to provide dosimetry and monitoring during radiobiological experiments.

N75 14423

Physical Conditions for Conducting Radiobiological Experiments in Beams of Accelerated Particles with High Linear Energy Transfer

In the USSR the majority of radiobiological experiments is conducted in multiple charge ion beams on the UMZI-300 cyclotron of the Laboratory of Nuclear Reactions of the Joint Institute of Nuclear Energy Research in Dubno. Basic parameters of the cyclotron are given in [2].

Parameters of accelerated ion beams are given in Table 39.

TABLE 39

CHARACTERISTICS OF ION BEAMS ACCELERATED IN THE UMZI-300 CYCLOTRON

Ions	A/z	Intensity of internal beam, μA	Intensity of extracted beam, μA	Energy of extracted ions Mev/nucleon
N_{14}^{+3}	4.67	200	15-20	11.45
B_{10}^{+2}, N_{15}^{+3}, Ne_{20}^{+4}, Ar_{40}^{+8}	5.00	150	15-20	10.0
O_{16}^{+3}	5.33	150-200	20-30	8.75
B_{11}^{+2}, Ne_{22}^{+4}	5.50	120-130	25-35	8.25
Ar_{40}^{+7}	5.71	5-10	1.5-2.5	7.68
C_{12}^{+2}, O_{18}^{+3}	6.00	120-250	40-80	6.95
N_{20}^{+3}, Ar_{40}^{+6}	6.66	30-150	15-50	5.62

Transport of the accelerated ion beam from the cyclotron (1) to the experimental unit (14) is shown in Fig. 34, a. Used to transport the beam are three quadrupole lenses (3), (8) and (12), three current collectors (4), (7) and (13), a vacuumized ion conductor (2), (9) and (11) and two rotating magnets RM (5) and (10).

Radiobiological experiments with radiosensitive biological objects require that the average rate of the absorbed dose lie in the $0.1-10/rad^3sec$ interval. For example, for Ne^{+4}_{20} ions with specific energy transport of ~6000 Mev/g·cm the ion current density is $10^3 sec^{-1}cm^2$ with a dose rate of 10^{-1} rad/sec. With a radiation field of 10 cm^2 a current of 1.6×10^{-9} μA corresponds to this flux. This indicates the necessity of reducing the intensity of the ion beam extracted for radiobiological experiments approximately 10^9 times while preserving its stability.

The use of the primary beam scattering method in obliquely located foil with radiation of biological objects in a field of scattered particles [28] showed that this reduces the intensity approximately 10^8 times, but in this /156
case unevenness of flux density through a cross section of the radiated object is significant (the coefficient of unevenness was 1.5-2) and the contribution of secondary radiation in the absorbed dose is 20%.

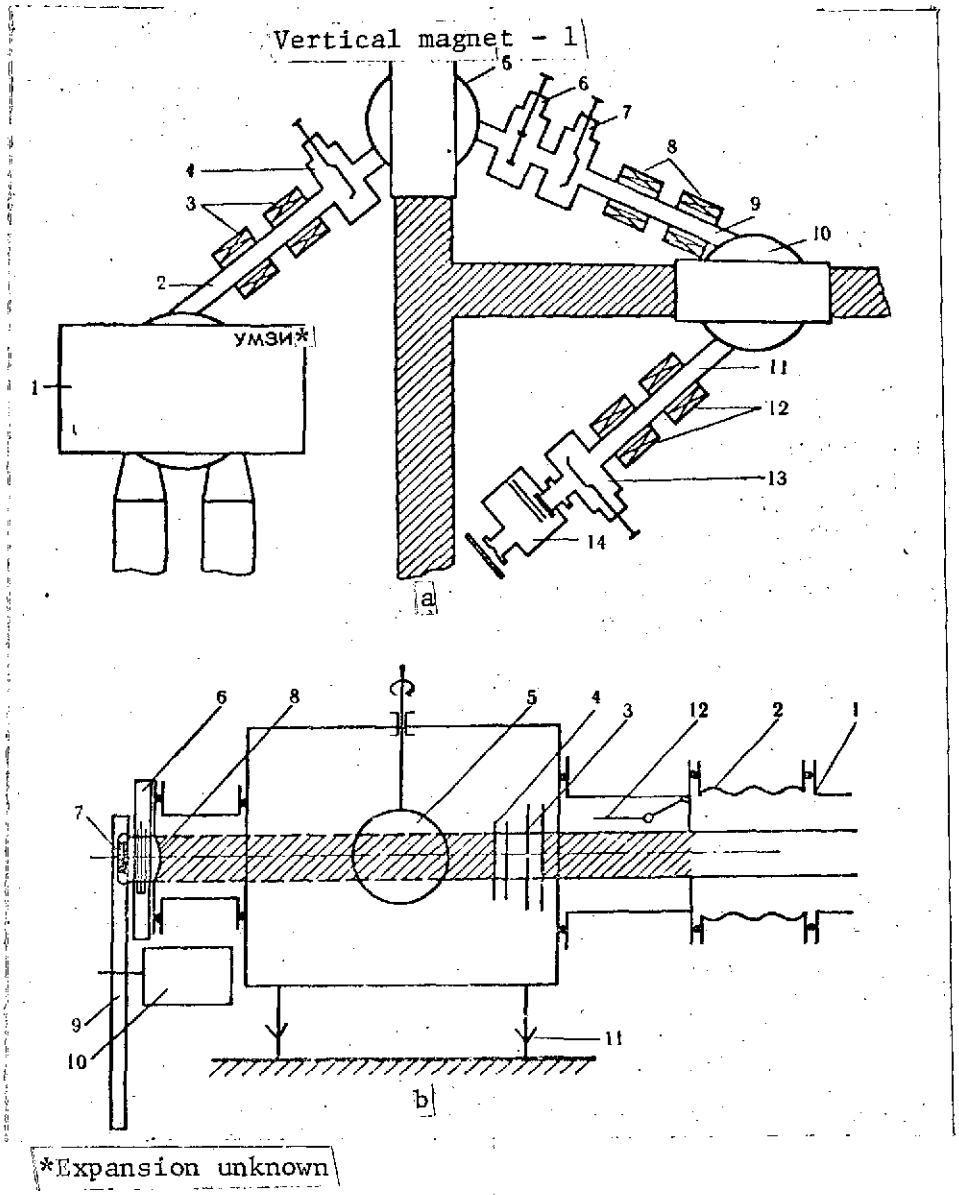

Figure 34. Diagram of experimental equipment (b) and its placement along the accelerator track (a).

Several methods have been considered for reducing the intensity of radiation of biological objects in a direct beam. Suggested as basic and used is the method of separating ions according to charges with a magnetic field after the ion beam has passed through a narrow target ("recharge method") [29].

Experiments conducted with Ne_{22}^{+4} ions showed the extensive possibilities /157
of this method. Ions are transmitted through aluminum foil (6) 10 μμ thick and deflected by a magnet (10)(see Fig. 34, a). Ion current was measured by a collector placed at the end of the ion conductor. Results of the experiment are given in Fig. 35. From this it is evident that conversion from charge $z = +6$ is accompanied by a 10^4-fold change in ion current.

In combination with defocusing by electromagnetic lenses placed on the beam extraction track and partial change in accelerating voltage, the "recharge" method made it possible to obtain the stable low-intensive ion beams necessary for conducting radiobiological experiments with radiosensitive objects.

When accelerated ions are discharged from the cyclotron in the absence of focusing lenses, the ion beam broadens in proportion to its progress in the ion conductor. The presence of recharge foil between the magnets increases the divergence of the ion beam because of ion scattering in it and range straggling as the result of which ions with different energy have different angles of rotation in the magnet.

Results of the experiments showed that with a diameter of the radiation field equal to or less than 2.5 cm, coefficient K_r of unevenness of the ion flux density through this field is at least 1.1 for differet ions from B_{10}^{+2} to Ar_{40}^{+8} with energies of 10 Mev/nucleon. By K_r is understood the ratio of maximum density of ion flux to its average value through the radiation field.

As a result of these studies it was possible to design and build equipment for radiobiological experiments in accelerated ion beams (see Fig. 34, b).

The vacuum chamber of the unit is adjusted with reference to the beam with the help of collector (5) using support screws (11) and bellows (2) attached to the ion conductor (1). With gate (6) open the ion beam is conducted to the atmosphere through an 8-10 μμ thick lavsan exit port (8). Drive (10) of cassette disk (9) with 12 places for biological objects (7) makes it

possible to move the object into radiation position remotely. In case of a break in the lavsan exit port a vacuum valve (12) is triggered. At the moment of radiation the current collector (5) is withdrawn from the beam.

Because the UMZI-300 cyclotron is an accelerator with unregulated particle energy this unit makes it possible to conduct radiobiological experiments in beams with various energy. Ion energy is altered by aluminum filters 1-99 mg/cm^2 thick arranged on disks (4). The filters are moved remotely by electric motors.

In a number of cases the ion beam with large dose rates was monitored by a so-called vacuum chamber (3)(see Fig. 34, b), which has two aluminum electrodes 1.25 mg/cm^2 thick; a positive potential is fed into one. The chamber uses electron emission from the metals as the ions pass through them. Typical volt-ampere characteristics of the chamber, located in the vacuum volume of the unit, are given in Fig. 36, b, which shows that even at 15 V saturation is observed in the collected current. The differential curve indicates that the maximum amount of emission electrons has energy in the area of 1.5 eV. Because of the absence of the phenomenon of recombination in the "vacuum" chamber, the /158
course of the saturation curve does not depend on the intensity of the ion beam. Dependence of the collected current on the state of the surface of electrodes, depth of vacuum and low sensitivity of the chamber do not allow it to be considered sufficiently reliable or a universal monitor in radiobiological experiments. Because of this, the chief monitor in the entire dose range from 0.1 - 1000 rad/sec. was an ionization air transfer plane-parallel chamber. This chamber has two high voltage lavsan electrodes 0.5 mg/cm^2 thick covered with a layer of aluminum 200-300 Å thick and a central collecting electrode of lavsan, aluminized on both sides.

An estimated calculation of the coefficient of collection f of the ionization chamber can be made in assuming uniform distribution of the space charge in the chamber [63]. However, column reconstruction of ions is not taken into consideration. It can be calculated for the case when there exists

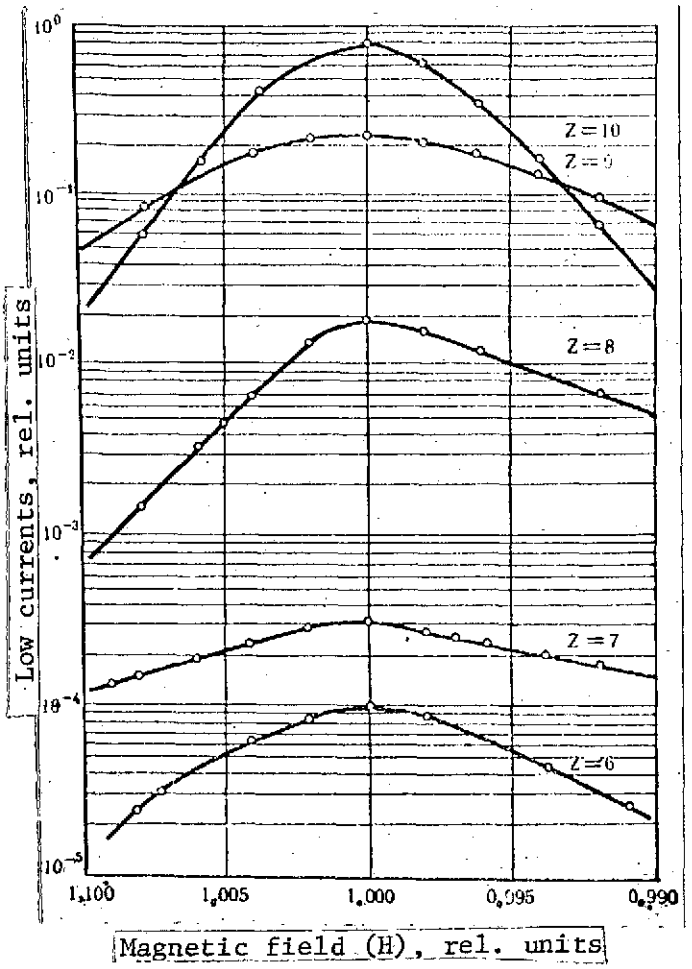

Figure 35. Dependence of the current in the collector of the experimental unit on magnetic field H of a rotary magnet and Z-ion charge entering the experimental unit.

a certain angle between the direction of the axis of the ion beam and the vector of the collecting field intensity [66]. When the angle is zero (as in our case) there are no approved methods of calculation and, therefore, experimental study of the characteristics of the chamber is necessary.

With the use of a "vacuum" chamber as a second monitor, volt-ampere characteristics of the ionization chamber were obtained with average dose rates of P = 10 – 1000 rad/sec for C^{12} ions ($d\frac{dE}{dx} - 3000$ Mev$\cdot g^{-1}cm^2$) and Ar^{40} ions ($\frac{dE}{dx} = 2 \cdot 10^4$ Mev$\cdot g^{-1}cm^2$) with energy of 4 Mev/nucleon (see Fig. 36, a). Weak dependence of current on dose rate in the curves indicates that column recombination is the basic process leading to reduction of the current recorded by the /159

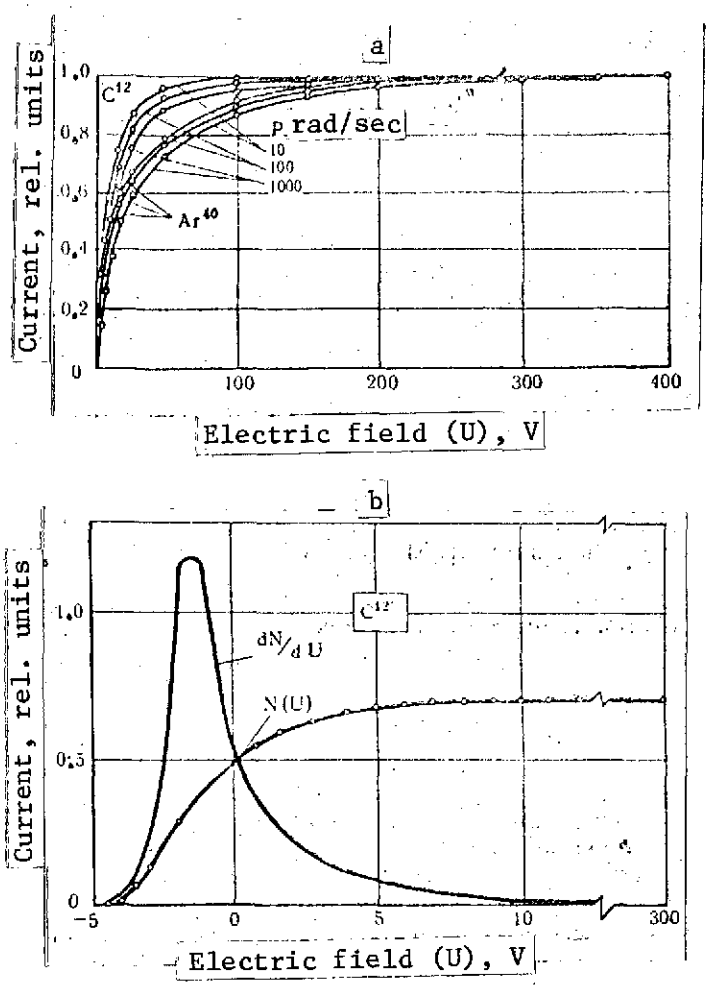

Figure 36. Volt-ampere characteristics of ionization transfer air plane-parallel chamber for C^{12} and Ar^{40} ions with rate of dose absorbed in the air of P = = 10-100 rad/sec (a) and vacuum chamber for a C^{12} ion with E/A = 4 Mev/nucleon (b).

chamber. Also observed is the pronounced effect of increased ion recombination with a rise in specific energy transfer. Analysis of these curves shows that with collecting voltage of 400 V, curves of current-voltage dependence reach a plateau making it possible to speak of practically complete ion collection in the chamber.

In experiments on C^{12} and Ar^{40} ion beams, with changes in the angle between the axis of the chamber and the direction of the beam from 0 to 20° and with v = 400 V, no increase was found in the current of the chamber with an increase of angle. This indicates that the chamber operates in regime of saturation.

In a radiobiological experiment, the chamber operated with collection voltage of 400 V and was placed directly in front of the biological object as close as possible to it. The current of the chamber was measured by an electrometer; the amplified current was converted to current impulses with a frequency proportional to the amplitude of the current at that moment of time. Impulses were fed to the intake of the coder which controlled the radiation process in accordance with a given program. /160

The ionization chamber used as a monitor was at the same time a dosimeter; the connection between the charge collected from the chamber and absorbed dose D in the biological object was described as follows:

$$D = \frac{S_m \cdot 10^5 \cdot \varepsilon k}{d \cdot A \rho_{0\,air} \frac{T_0}{T} \cdot \frac{P}{P_0}},$$

where d = 0.1000 is inter-electrode distance, cm; A is the sectional area of the beam passing through the chamber, cm^2; $\rho_{0\,air}$ is the density of the air at Kelvin scale temperature and pressure P_0, i.e. $\rho_{0\,air} = 1.205 \cdot 10^{-3}$ g/cm^3 with $T_0 = 273°K$ and $P_0 = 760$ bar; $\varepsilon = 35.2$ eV is energy for the formation of a pair of ions in air [95]; $S_m = 1.15$ is the ratio of mass retarding capacities of tissues and air with ion energy E; $K = \frac{\left(\frac{dE}{dx}\right)_{ob}}{\left(\frac{dE}{dx}\right)_{cham}}$ is the ratio of specific losses of energy by the ion on the surface of the object and in the center of the ionization chamber, determined by reduction of ion energy on the way from the ionization chamber to the object. In case of uneven radiation of the surface of the biological object, by D is meant the average absorbed dose on the surface of the biological object.

Maximum relative error of measurement by such a chamber of the absorbed dose in the tissue of the object, connected with the uncertainty of values in expression (1), is not over ±5%. Energy spectra of ions, depending on the depth of the biological object, were measured by a semiconductor drift detector with energy resolution of 1% for different layers of lavsan simulating

biological tissue.

Because of straggling of ion ranges the energy line widens as the ion progresses deeper into the object, but this widening is significant only at the end of the ion range where $\Delta E/E \approx 25\%$. In areas of depth from 0 to 10 mg/cm^2 $\Delta E/E \leq 5\%$. For heavier ions this value will be even less.

Thus, it can be concluded that the uncertainty in $\frac{dE}{dx}$ as a whole is determined by its change as the ion penetrates into the biological object with initial ion energy monoline s leaving the accelerator (practically, the relative energy widening of exiting ions is not worse than 1%). Depth distribution of the absorbed dose in the radiated biological object, inviview of the good energy homogeneity of the beam, its monodirection and small scattering cross sections in atoms of the absorbed substance, is practically determined by the dependence of $\frac{dE}{dx}$ on energy of the decelerating ion at the end of the range (0-5 mg/cm^2) — by ion range straggling.

With the use of these works [75] on ranges, specific energy losses of B_{11} and C_{12} ions in lavsan were calculated and at the same time a Bregg curve in lavsan was plotted, making it possible to come to the following conclusions: /161

1. The effect of range straggling on the formation of absorbed dose is essential for light and energetic ions and at the end of the ion range, i.e., in the area of residual ranges 7-10 mg/cm^2.

2. With a change in the energy spectrum of ions hitting the biological object there is a deformation of depth distribution of the dose.

3. Experimental data obtained with $\Delta \frac{E}{E} = 1\%$ agrees closely with the calculated course of D(h) with $\Delta \frac{E}{E} = 1\%$.

Thus, depth distribution of the absorbed dose from a monodirectional monoenergetic ion beam is practically completely presented by the dependence of

$\frac{dE}{dx}$ on the depth of the object which can be obtained using these works [75].

Calculation of the contribution of secondary radiation consisting of charged particles and neutrons in the most unfavorable case showed that this contribution does not exceed 2% of the absorbed dose expressed in rads.

In the GDR, radiation of radioresistant biological objects is being conducted in the cyclotron of the Central Institute of Nuclear Research (Rossendorf) with x-particles, deuterons and protons with average energy of 27, 13.5 and 6.75 Mev [57, 62].

Uniformity of intensity through the radiation field provided during the radiation process was quite high, as dimensions of this field (about 0.7 cm^2) of a rectangular shape were less than the cross section of the beam of accelerated particles. Accurate adjustment was provided by indicators which changed color under the effect of radiation. Later a special transfer chamber was designed and built for this purpose which was located between the exit port of the particle beam and the disk with the biological objects. The presence of three measuring electrodes in this chamber made it possible to measure the distribution of intensity through the width of the beam.

Biological objects in solid phase were radiated outside the vacuum part of the unit on a rotating disk. A unit for radiating biological solutions was designed and built in which the radiated liquid passes under pressure through a nozzle 170 μμ in diameter perpendicularly to the beam of accelerated particles.

The dose produced for the biological object was measured in an ionization chamber, a Faraday cylinder and a ferrosulfate dosimeter. The measuring volume of the ionization chamber was rectangular in shape measuring 0.5 x 1.3 x x 0.5 cm^3. Collecting electrodes were made of graphitized teflon.

Conditions of saturation with 30,000 rad/sec. were provided by applied voltage of 2500 V. A similar mica port in the chamber was 3 mg/cm^2 thick.

The ferrosulfate method is used only in radiating liquids. In such case the ferrosulfate solution passes through the radiation unit instead of the biological liquid under the same conditions. Doses determined with the ferrosulfate solution and measured in the ionization chamber differ from each other not more than ±10%.

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Isotope a-irradiators for Radiobiological Research /162

In practice most often encountered is the case of radiation when the α-source in the form of a flat disk with radius R_{source} and axially-located biological object, also in the form of a flat disk with radius r_0 and thickness of h_0, are separated by a layer of gas with thickness H and density ρ_0. Frequently the biological object can be covered by a polymer film with minimal thickness C for protection against radioactive contaminants. The diagram for calculation is given in Fig. 37.

Evenly distributed through the cross section of the source is an α-active preparation with specific activity A. It is assumed that the source is thin and, therefore, angular distribution of α-particles emitted from the active layer can be considered isotropic. In this case, the density of the flux of α-particles at the point with polar coordinates R and ψ will be:

$$N(r,h)=\frac{A}{2\pi}\int_0^{\pi}d\psi\int_0^{R_{source}}\frac{RdR}{R^2+(H+c+h)^2+r^2-2rR\cos\psi} \qquad (1)$$

The expression for the absorbed dose rate R in elementary volume dV of the substance with density ρ_0 when it is radiated with contaminated particles is described as follows:

$$P=\frac{A}{2\pi\rho_0}\int_0^{R_{source}}dR\int_0^{\pi}\frac{dE}{dx}(R,\psi,H,h,r)\frac{Rd\psi}{R^2+(H+c+h)^2+r^2-2rR\cos\psi} \qquad (2)$$

Dose D at point M (r, h) taking into account that $T_{1/2} >> t$ is:

$$D = P \cdot t. \tag{3}$$

Expression (2) was derived for one monoline of α-particles. In practice, because of the final thickness of the source and the presence of several lines of α-particles, an energy spectrum is observed which for a "thin" source can be considered normal and described in the form:

$$W(E) = \frac{1}{\sqrt{2\pi}\,\sigma_E} \exp\left\{-\frac{(E-E_0)^2}{2\sigma_E^2}\right\}, \tag{4}$$

it is known that $\int_{-3\delta}^{+3\delta} W(E)\,dE = 0{,}998$. Converting to recording expression W(E) by halfwidth Δ_E of energy distribution in its halfheight, we obtain:

$$W(E) = \frac{0{,}47}{\Delta_E}\left\{-\frac{0{,}693\,(E-E_0)^2}{\Delta_E^2}\right\},$$

where E_0 is the most probable energy of α-particles. If it is assumed that the angular distribution of α-particles of the source does not depend on its energy, then expression (3) can be written finally in the form:

$$D = \frac{0{,}075\,At}{\rho_0\,\Delta_E} \int_{E_0-2{,}55\Delta_E}^{E_0+2{,}55\Delta_E} dE \int_0^{\pi} d\psi \int_0^{R_{source}} \frac{dE}{dx}(R, \psi, H, h, r)\,\frac{R\,dR}{L^2(R,\psi)}\,e^{-\frac{0{,}693(E-E_0)^2}{\Delta_E^2}} \tag{5}$$

where

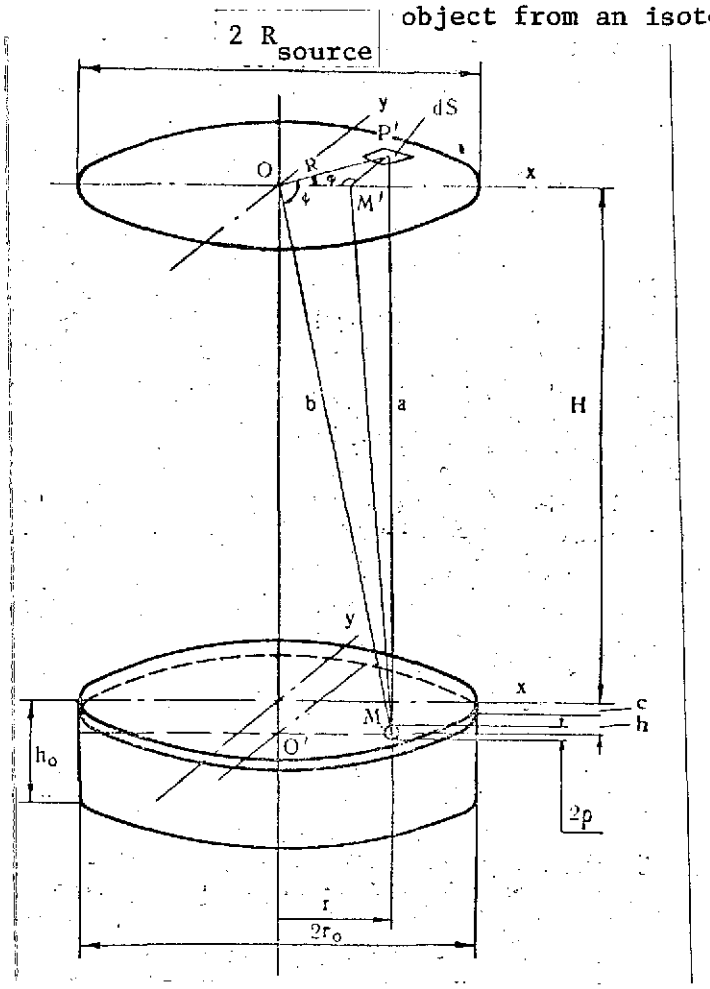
$$L^2(R, \psi) = R^2 + (H + c + h)^2 + r^2 - 2rR\cos\psi.$$

Values of N (r,h) and D (r, h) were calculated numerically with the use /163 of these works [96] on ranges and specific losses of energy of α-particles for nitrogen, carbon, oxygen and argon which are in air, tissue and the polymer protective film. At the same time the energy of the α-particle was calculated at the place where the absorbed dose was determined, taking into account loss of energy in air, film and tissue. The level of energy was determined by the specific loss of energy of the α-particle arriving from the point of the source P^1 with coordinates R, ψ to point M (r, h) of the biological object.

According to the known spectrum of specific losses of energy, the average specific losses of energy $\frac{dE}{dx}$ and dispersion of spectrum σ^2 were determined:

$$\overline{\frac{dE}{dx}}(r, h, H) = \frac{\int_{E_1}^{E_2} dE \int_{S_{source}} \frac{dE}{dx}(E, S)\, N(S)\, W(E)\, dS}{\int_{E_1}^{E_2} dE \int_{S_{source}} N(S)\, W(E)\, dS}, \qquad (6)$$

Figure 37. Diagram for calculating the absorbed dose in a biological object from an isotopic α-source.



$$\sigma^2(r, h, H) = \frac{\int_{E_1}^{E_2} dE \int_{S_{source}} N(S)\, W(E) \left[\frac{dE}{dx}(r, h, H, S, E) - \frac{\overline{dE}}{dx}(r, h, H)\right]^2 dS}{\int_{E_1}^{E_2} dE \int_{S_{source}} N(S)\, W(E)\, dS}, \quad (7)$$

/164

where S_{source} is the area of the source; N(S) is the number of α-particles hitting point M (r, h, H) from the element of source dS; E_1 and E_2 are equal to $E_0 - 2.55\Delta_E$ and $E_0 + 2.55\Delta_E$, respectively.

Relative dispersion Δ was determined from expression

$$\Delta = \frac{\sigma}{\frac{\overline{dE}}{dx}}. \quad (8)$$

The coefficient of inequality K_r of distribution of flux density of α-particles through a radiation field with radius r_0 is found as follows:

$$K_r = \frac{N_{max}(H, r=0)\,\pi r_0^2}{\int_0^{r_0} N(r, H)\, 2\pi r\, dr}. \quad (9)$$

Average absorbed dose $\overline{D}$ is determined as

$$\overline{D} = \frac{\int_0^h dh \int_0^{r_0} D(r, h)\, r dr}{r_0^2 h}. \quad (10)$$

Then coefficient K_V of the inequality of distribution of absorbed dose through the volume of the biological object is

$$K_v = \frac{D(r=0, h_{max})}{\overline{D}},$$

where h_{max} is the depth at which dose D reaches maximum.

Selection of the radioactive isotope for preparing the α-source is determined by the following requirements:

a) sufficiently long half-life period $T_{1/2}$;

b) maximum energy of emitted α-particles;

c) minimum widening of the energy spectrum of α-particles;

d) maximum possible specific activity for obtaining minimum thickness of the isotope layer on the backing;

e) minimum background of accompanying radiation;

f) validity of the isotope for preparing the source.

Taking into account the requirements listed in points "a" – "f", sources can be prepared from isotopes V^{232}, Pu^{238}, Pu^{239}, Am^{241}, Cm^{244} and isotopes with relatively short half-life periods: Po^{210} ($T_{1/2}$ = 138 days), Th^{238} ($T_{1/2}$ = 1.9 years) and Pu^{236} ($T_{1/2}$ = 2.7 years) [34].

The energy of α-particles emitted in the nuclear disintegration of these isotopes changes within narrow limits from E_α = 5.24 Mev (Pu^{239}) to E_α = 5.78 Mev (Cm^{244}). In our calculations we selected isotope Am^{241} which is characterized by the following parameters [35]: $T_{1/2}$ = 475 years; E_α = 5.379 Mev (1.42%), 5.433 Mev (13.6%) and 5.476 Mev (84.2%) as well as several other lines with a total contribution of 0.78%; γ-radiation accompanying the emission of α-particles of basic lines has energy: 26, 33, 60, 14 and 19 kev; specific activity is 0.32 Cu/g. /165

For the purpose of studying the dependence of N and K_r on radiation conditions, variant calculations were conducted for the case of $A\pi R^2_{source}$ = const for various H from 0.2 to 1.5 cm and R_{source} from 0 to 2 cm with a fixed radius of the radiation field r_0 = 0.5 cm. Results of calculations are given in Fig. 38. As follows from Fig. 38, a, with a given radius of the radiation field, increase of radius R_{source} of the source and height H leads to a reduction of the inequality in distribution of flux density of α-particles through the radiation field; with fixed coefficient K_2 with increased R_{source}, it can assume ever decreasing values.

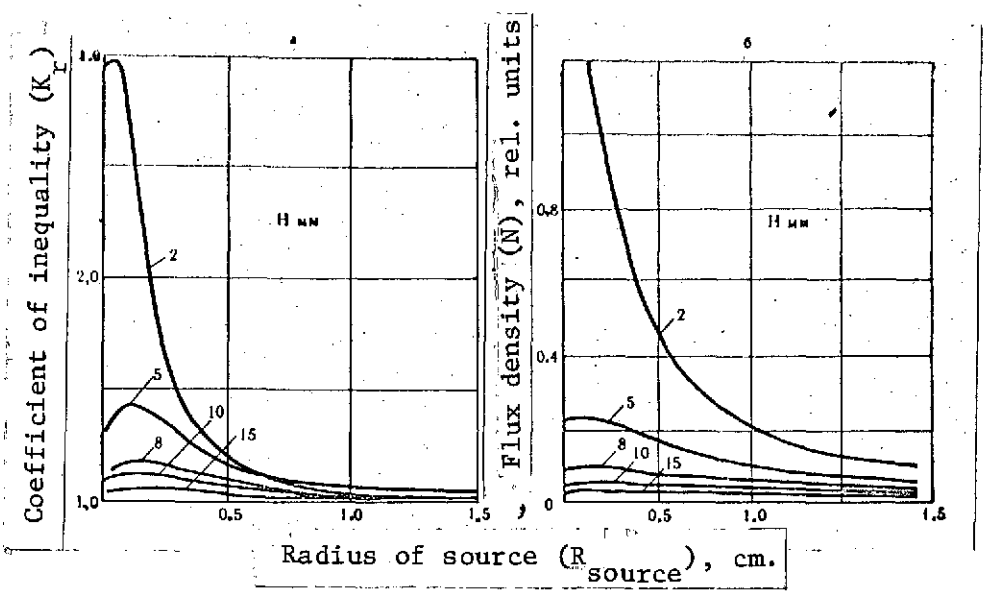


Figure 38. Dependence of the coefficient of inequality R_r of the radiation field with radius $\tau_0 = 0.5$ cm on R_{source} and H with a constant value $A = \pi R^2_{source}$, where A is the specific activity of α-source (a) and the dependence of flux density of α-particles in the center of the biological object on the radius of source R_{source} (b) and distance H between the source and the biological object.

From the curves in Fig. 38, b, it follows that from the point of view of radiation use of a radioactive isotope to produce a given flux density and reduce error in the absorbed dose during radiation because of inaccurate knowledge of the values of H and R_{source} the range of changes in H must be 0.5-1 cm and in R_{source} = 0.5-1 cm.

Based on these considerations we made calculations of the absorbed dose rate R, average specific loss of energy $\frac{dE}{dx}$, $\frac{1}{\rho_0}$ and relative dispersion of the spectrum of specific losses of energy of α-particles Δ at points H equal to 0.5, 0.8, 1.0 and 1.5 cm, with r equal to 0.0, 0.3 and 0.5 cm, for various depths of h of the biological object, with $A = 10^7$ cm^{-2} sec^{-1} (E_α = 5.48 Mev, R_{source} = 0.5 cm). Results of the calculation are given in Table 40 and in Fig. 39.

TABLE 40

EXPERIMENTAL AND CALCULATED CHARACTERISTICS OF α-SOURCE /166

R_{source}=0.5 cm, Δ_E=0.01 Mev and $A=0.32\cdot10^7 cm^{-2}sec^{-1}$ (E_α=5.48 Mev)

H, cm	r, cm	h, mg/cm²	0,0	0,5	1,0	1,5	2,0	2,5	3,0
		Δ, %							
		calc.	0,74	0,78	2,0	3,4	6,4	12,1	14,5
		exp.	1,1	1,3	2,2	4,5	7,6	13,0	—
	0	$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm²/g							
		calc.	932	1012	1130	1301	1597	2248	718
		exp.	210	1090	1090	1240	1440	1760	—
		R, rad/sec							
		calc.	3,77	4,18	4,71	5,48	6,72	9,28	2,98
		exp.	—	4,0	4,3	4,7	5,0	6,9	5,5
		Δ, %							
		calc.	1,42	1,84	4,5	8,5	18,0	54,4	142
		exp.	1,5	—	4,6	—	—	—	—
0.8	0.3	$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm²/g							
		calc.	938	1019	1157	1353	1762	1702	765
		exp.	910	—	1110	—	—	—	—
		R, rad/sec							
		calc.	3,43	3,90	4,30	5,08	6,77	6,45	2,88
		exp.	—	3,6	3,9	4,4	5,2	5,5	4,0
		Δ, %							
		calc.	2,00	3,86	7,14	15,4	39,4	84,5	223
		exp.	2,2	—	7,8	—	—	—	—
0.8	0.5	$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm²/g							
		calc.	949	1040	1201	1489	1685	1300	376
		exp.	930	—	1140	—	—	—	—
		R, rad/sec							
		calc.	3,13	3,55	3,94	4,89	5,41	4,32	1,24
		exp.	—	3,2	3,6	4,1	4,5	4,0	2,5
		Δ, %							
		calc.	0,69	0,99	1,71	2,85	5,32	7,12	—
		exp.	0,8	1,1	2,0	3,0	5,0	8,5	—
1.0	0	$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm²/g							
		calc.	963	1032	1179	1373	1724	2464	—
		exp.	960	1040	1150	1290	1500	1880	—
		R, rad/sec							
		calc.	2,82	2,91	3,33	3,91	4,93	6,94	—
		exp.	—	2,8	3,0	3,4	3,9	4,6	—

TABLE 40

Continued

H, cm	r, cm	h, mg/cm^2	0,0	0,5	1,0	1,5	2,0	2,5	3,0
1.0	0.3	Δ, %							
		calc.	1,28	2,81	3,92	6,68	14,4	56,7	—
		exp.	1,6	—	4,0	—	—	—	—
		$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm^2/g							
		calc.	966	1041	1189	1393	1784	2002	—
		exp.	940	—	1170	—	—	—	—
		R, rad/sec							
		calc.	2,72	2,80	3,19	3,75	4,85	5,50	—
		exp.	—	2,7	3,0	3,4	3,9	4,2	—
	0.5	Δ, %							
		calc.	1,81	4,12	6,16	12,0	31,8	93,4	—
		exp.	1,9	—	6,0	—	—	—	—
		$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm^2/g							
		calc.	978	1068	1249	1520	1872	1261	—
		exp.	980	—	1160	—	—	—	—
		R, rad/sec							
		calc.	2,38	2,53	2,94	3,58	4,40	2,99	0
		exp.	—	2,5	2,7	3,2	3,4	3,3	1,7
		Δ, %							
		calc.	0,70	0,98	1,58	2,79	4,9	—	—
		exp.	0,8	1,2	1,7	2,9	5,4	—	—
		$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm^2/g							
		calc.	1034	1179	1366	1693	2433	—	—
		exp.	1020	1130	1280	1500	1850	—	—
		R, rad/sec							
		calc.	1,38	1,61	1,81	2,29	3,11	0	0
		exp.	—	1,5	1,7	1,9	2,2	2,9	0
1.5	0.3	Δ, %							
		calc.	1,50	1,91	3,21	6,34	15,6	—	—
		exp.	1,7	—	3,5	—	—	—	—
		$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm^2/g							
		calc.	1041	1190	1390	1758	2384	—	—
		exp.	1030	—	1300	—	—	—	—
		R, rad/sec							
		calc.	1,32	1,49	1,86	2,29	3,04	0	—
		exp.	—	1,5	1,65	1,9	2,3	1,5	0

/167

TABLE 40

Continued

/168

H, cm	r, cm	h, mg/cm^2	0,0	0,5	1,0	1,5	2,0	2,5	3,0
	0.5	Δ, %							
		calc.	2,52	2,89	5,18	11,0	58,5	—	—
		exp.	2,5	—	7,0	—	—	—	—
		$\frac{1}{\rho_0}\frac{\overline{dE}}{dx}$, Mev·cm^2/g							
		calc.	1058	1210	1431	1879	1788	—	—
		exp.	1040	—	1330	—	—	—	—
		R, rad/sec							
		calc.	1,31	1,48	1,73	2,35	2,20	0	—
		exp.	—	1,4	1,65	1,9	2,0	1,3	0

From these data it follows that:

1. With axial location of the source and the biological object, a direction toward equalizing the field of absorbed doses is traced along a radius with height H. Absolute value of the dose in the center of the surface of the object is reduced according to law $H^{-\gamma}$ where $\gamma \leq 2$.

2. With increased depth h the dose field reaches maximum, which with an increase of h first shifts to the depth of the biological object and then to its surface. This is due to two competing processes: increase in the average loss of energy of α-particles, connected with reduction of their energy, and reduction in their flux density as h increases.

3. Relative dispersion $\Delta \frac{dE}{dx}$ makes it possible to judge the shape of the spectrum occurring in the biological object. In Fig. 39 this dependence is given in the form of a band determined by relative dispersion with r = 0 (lower limit) and r - 0.5 cm (upper limit). A tendency toward increased dispersion is traced distinctly with increased depth h of the biological object, connected with the increased difference in ranges of α-particles with their passage through air and tissue as H is reduced and r or h is increased.

For the purpose of verifying these results, experimental measurements were made of the absorbed dose rate and energy spectrum of α-particles depending on

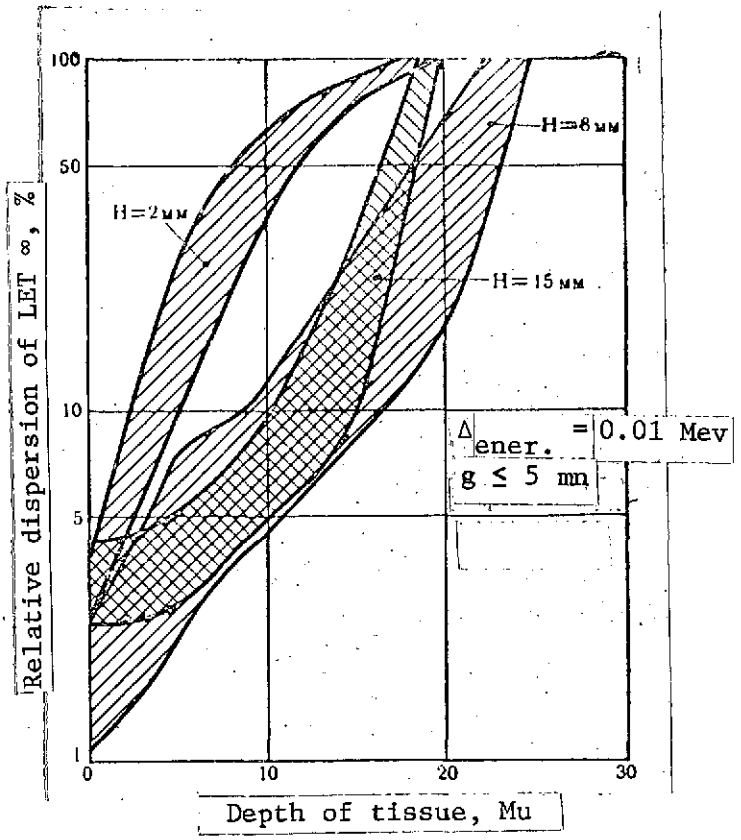


Figure 39. Relative dispersion of Δ-spectrum of specific losses of energy of α-particles, depending on coordinates r and h of the biological object for different H with R_{source} = 0.5 cm, $\Delta_{ener.}$ = = 0.01 Mev. Upper limits of the bands correspond to r = 0, lower to 5 = = 0.5 cm.

values of H, r and h. Lavsan was used as tissue-equivalent material in which the ratio of mass specific losses of energy of α-particles to losses in tissue is constant and practically equal to one in a wide range of α-particle energy. The energy of α-particles was measured with a semiconductor surface-barrier detector whose surface incorporated an aluminum diaphragm 0.1 cm in diameter. Absorbed dose rate was measured by a specially designed air ionization chamber with plane-parallel electrodes placed 1.15 mm apart. The exit port of the chamber was made of aluminized lavsan 0.5 mg/cm^2 thick. The chamber was operating in a saturation regime with $\overline{p} \approx 10$ rad/sec even at a voltage of V = = 15V. On the surface of the exit port (2 cm in diameter) there were diaphragms whose openings occupied various locations along the radius of the exit /169 port. Ionization currents were, therefore, measured at various sites in the radiation field. The current of the chamber was measured with an electrometer with a vibrocondenser. Experimental results Δ, $\frac{1}{\rho} \cdot \frac{dE}{dx}$ and R for various

layers of lavsan for an α-source with R_{source} = 0.5 cm and A = $0.32 \cdot 10^7 \cdot cm^{-2} sec^{-1}$. ($E_\alpha$ = 5.48 Mev) are given in Table 40.

As seen from these data, relative dispersion $^{dE}/_{dx}$ on the average coincides with accuracy at least 20%, absorbed dose rate R with accuracy of at least 15% with all H changing in the H range from 0.8 to 1.5 cm and h from 0 to 1.5 mg/cm^2. The observed deviation of experimental values $^{dE}/_{dx}$ and absorbed dose rate toward a reduction from calculated values is due, evidently, to measurement error (10-15%) with regard to the density of the lavsan film used in the experiment and overstated values of calculated $^{dE}/_{dx}$. Therefore, for example, with H = 1.5 cm and h = 2.5 mg/cm^2, measured dose rate is 2.9 rad/sec. and the calculated value is zero.

On the basis of these studies, an experimental unit was designed making it possible to radiate several sources in a given gaseous environment. The distance between surfaces of the object and the source are taken as 0.8 cm and can be changed ±0.2 cm. The radiation field is not over 0.5 cm. In this case the coefficient of inequality $K_F \leq 1.10$. Thickness of the object h_0 must /170
not be over 1.5 mg/cm^2 because of the excessive increase of relative dispersion of the Δ-spectrum $^{dE}/_{dx}$ (over 30%) at such depths and it is, therefore, desirable that $h_0 \leq 1$ mg/cm^2 (then $\Delta \leq 10\%$). Change in $^{dE}/_{dx}$ in this case ($h = 1$ mg/cm^2) is insignificant: 930-1130 $Mev \cdot g^{-1}cm^2$ with r = 0.0 cm and 950-1200 $Mev \cdot g^{-1}cm^2$ with r = 0.5 cm. Thus, the average specific losses of energy in the object are also given with accuracy at least 10%. Change in the absorbed dose rate caused by using an α-source with necessary specific activity makes it possible to maintain standard radiation conditions, which improves reproducibility of the results. Thanks to the use of a source with R_{source} = = 0.5 cm and A = $0.32 \cdot 10^7 cm^{-2} sec^{-1}$, absorbed dose rates about 20 rad/sec (for Am^{241}) can be obtained. There are no theoretical limitations for radiation with dose rates of 100-0.01 rad/sec or less in preparing corresponding α-sources.

N75 14425

Microdosimetry and the Radiation Danger of Cosmic Rays

The radiation danger of space flights is due, as is known, primarily to intensive proton fluxes accompanying solar flares and heavy multiple charged ions in galactic cosmic radiation. Evaluation of the biological effect of these kinds of rays is based on calculations of tissue dose, taking into account the formation of secondary radiation in shielding and tissue. The essential role in such calculations is played by the dependence of the factor of quality on linear energy transfer (LET) of these charged particles.

Recommendations concerning the dependence of the quality factor on LET are based on radiobiological experiments carried out on many biological systems using various kinds of rays, including heavy ions with energies in the 1-10 Mev/nucleon range. Justification of the results of these experiments, as well as their extrapolation to other energies, requires development of a model description of the radiation effect of charged particles on biological microstructures.

The majority of works on the quantitative evaluation of radiobiological effects by calculation methods which have been carried out until now compute the probability of the studied radiobiological effect with radiation in dose D:

$$g(D) = \int_0^\infty f(z, D)\, G(z)\, dz, \qquad (1)$$

where z is the energy absorbed in some sensitive volume of the biological object (or other proportional value); f(z, D) is probability density of absorption of energy z with radiation dose D;

G(z) is the probability of realization of the studied radiobiological effect with absorption in its sensitive volume of energy z.

In calculating g(D) according to formula (1) it is essentially assumed that:

a) radiation is instantaneous, i.e., radiation injury developing after transmission of dose D can be disregarded:

b) the distribution of absorbed energy within the sensitive volume has no effect on the probability of realization of the studied radiobiological effect, i.e. G(z) does not depend on LET. /171

Reference works on determining g(D) used f(z,D) obtained with various degrees of approximation and G(z) conforming to various ideas of the development of radiation damage after radiation. For this we shall first make a comparative analysis of the methods used to calculate f(z, D) and then review the basic directions in selecting the kind of G(z).

Determining the density of distribution of absorbed energy in microvolumes of various shapes is, as is known, the basic item of study of microdosimetry. Experimental and calculation methods of microdosimetry make it possible to take into account not only fluctuations in the number of hits a charged particle makes in a microvolume corresponding to the dimensions of the biological object or its sensitive volume (the cell, its nucleus, bacteria, virus, DNA, etc.), to which, for example, the multi-impact theory is limited, but also other physical causes of fluctuation in energy transmitted to the microvolume, due to the discrete character of the interaction between radiation and the substance. Such causes in case of charged particles include fluctuations in the mean free path of a charged particle in the microvolume, the spread in number of ionizations and energy lost by the particle in each of these ionizations.

For charged particles which transmit the greater portion of energy to δ-electrons, the cause of fluctuations could also be the spread of distances from the trajectory of the particle to the microvolume, fluctuation in the number of δ-electrons formed near the microvolume, their energy, trajectory, etc. The last causes, as follows from [9, 10, 30, 71], are extremely important for high-energy multi-charged ions.

As the density of distribution of $f(z,D)$ is formed during the transmission of energy to the microvolume by several statistically independent particles, it is calculated on the basis of the density of distribution of $f_1(z)$, corresponding to a single act of energy transmission to the microvolume (one event of absorption). In connection with this, in microdosimetry a great deal of attention is given to calculated determination of $f_1(z)$ for microvolumes (basically spherical, but also flat) with dimension d changing in a range from 0.01 to 10 μμ.

Calculated methods of microdosimetry are distinguished primarily by how each of the phenomena caused by fluctuation of energy transmitted to the microvolume is calculated — on the average or stochastically.

The simplest method of calculation is based on the assumption that transmitted energy is proportional to the path of the particle in the microvolume. For a specific volume this method, as is known, gives triangular distribution. However, the assumption is true for heavy charged particles if:

1) the number of primary ionizations is high enough so that its fluctuation in the path within the microvolume is not taken into account, i.e., $\bar{\kappa}d \gg 1$, where $\bar{\kappa}$ is the average number of primary ionizations per unit of the path;

2) the range of a charged particle R is several times greater than the dimensions of the microvolume, i.e. $R > d$;

3) the radius of the track spread of the charged particle because of δ-electrons — ρ_{max} is several times less than the diameter of the microvolume, i.e., $\rho_{max} < d$.

Such a method of calculation makes it comparatively easy to show the effect of the shape of the sensitive volume on the kind of function of $f_1(z)$. /172

To illustrate the area of applicability of triangular density of distribution in Fig. 40 are plotted dependences $d(^{dE}/_{dx})$, satisfying conditions $\varkappa d = 100$, $R = 5$ and $d = 0.1\ \rho_{max}$ for particles with charges of 1, 2 and 6. The value of ρ_{max} we took from work [30] and z was calculated according to the Rutherford formula assuming that the minimum transmission of energy in a collision between the particle and an electron is 100 ev.

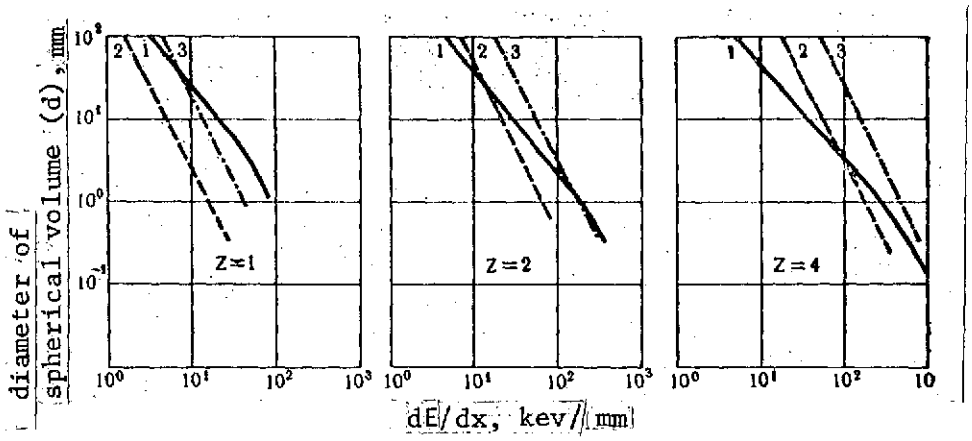


Figure 40. Graphs of the dependence of the diameter of a spherical microvolume d on dE/dx, satisfying conditions $xd = 500$, $P_{max} = 0.5\ d$ and $R = 10\ d$ for particles with charges z equal to 1, 2 and 4.

As seen from Fig. 40, triangular density of distribution, despite the simplicity of obtaining it, is applicable in an extremely limited area of microvolume sizes and values of $^{dE}/_{dx}$. It is important that the limitation in area of small volumes for particles with a charge greater than one is determined by the effect of energy transfer by δ-electrons.

The next step in comparison with the previous approximation is the approach which considers the fluctuation in the number of primary ionizations in the range of the charged particle in the microvolume. For a sphere the

density of distribution of f(z) in this approximation can be obtained in analytical form if it is assumed that the energy transmitted in each primary ionization is constant. In this case the probability that the particle will generate k primary ionizations within the sphere is:

$$p(k) = \int \frac{2ldl}{d^2} \frac{(\chi l)}{k!} e^{-\chi d} = \frac{2(k+1)}{(\chi d)^2}\left[1 - e^{-\chi d}\sum_{j=0}^{k+1}\frac{(\chi d)^j}{j!}\right] \quad (2)$$

and correspondingly $f_1(z) = p(z/z_0)$, $z_0 = \frac{dE}{dx}\Big/\chi$ is the average transfer of energy per one primary ionization. This approximation has a wide area of applicability for protons, as it is true with a fewer number of collisions in /173 a diameter — $\kappa \geq 50$.

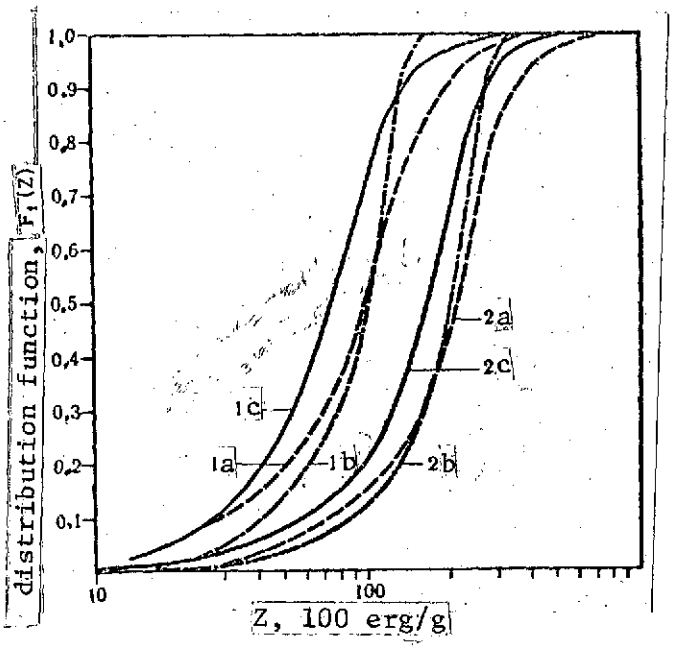


Figure 41. Function of distribution of local density of energy $F_1(Z)$ in one event of absorption, calculated in different approximations for spherical area 1 μμ in diameter.

Curves 1a, 1b and 1c — for protons with energy of 9 Mev; 2a, 2b and 2c — for protons with energy of 4 Mev; 1a, 2a — data from work [64]; 1b, 2b — calculated according to formula (2) (as Z_0 was taken the dose corresponding to release of energy of 35 ev in the microvolume); 1c, 2c — calculated according to Monte Carlo method taking into account transfer of energy by δ-electrons.

The fluctuation of energy transferred in primary ionization could not analytically be taken into account and in this case the Monte Carlo method is used [64]. Distribution function of a local dose for protons with energy of 4 and 9 Mev and for microvolumes with dimension $d = 1$ μμ obtained in work [64] are given in Fig. 41 along with distribution functions obtained according to formula (2). As can be seen, for protons with energy of 9 Mev, for example, average values of transferred energy are very slightly distinguished, but second moments differ markedly. Although results of work [64] are formally applicable with as low values of $\overline{\kappa d}$ as desired, low values of $\overline{\kappa}$ correspond to high energy of charged particles and, correspondingly, an important role is played by δ-electrons whose transfer of energy was not taken into consideration in this work. As a result, the method used in work [64] does not expand the limits of applicability of calculations according to formula (2) for α-particles, and in proton radiation with energy in tens of mega-electron volts is applicable for small microvolumes, but not less than several microns.

As the above outlined methods of calculating $f_1(z)$ and the results obtained by these methods do not take into consideration transfer of energy by δ-electrons, the change in dE/dx within the microvolume and the termination of ranges in the microvolume, there is doubt of the applicability to heavy recoil nuclei formed by neutrons, to low-energy recoil protons and to heavy multi-charge high-energy ions, i.e., to just those kinds of particles for which are recommended factors of quality greater than one. It must be noted that experimental methods of determining $f_1(z)$ are least applicable to such kinds of particles.

The simplest means of calculating the effect of energy transferred by δ-electrons can be used when the dimensions of the biological object and dE/dx are so small that $\overline{\kappa d} \lesssim 1$ and the probability of two δ-electrons from the same charged particle hitting the microvolume can be disregarded. This makes it possible to consider δ-electrons hitting the microvolume as statistically independent, i.e., independent primary particles with a given volume density of their development in the environment and within the volume. According to δ- /174

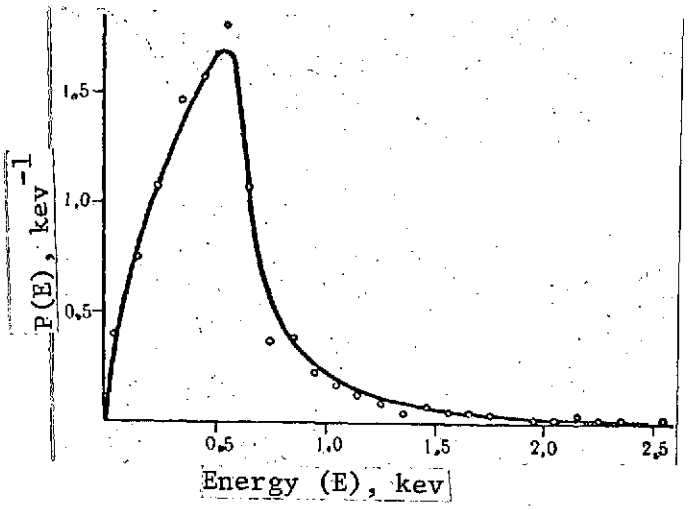


Figure 42. Spectrum of absorbed energy R(E) in a spherical microvolume 0.2 μμ in diameter from a surface cosine electron source with energy of 5 kev. Curve taken from work [11], points — results of calculation according to the program we developed (6000 histories).

electrons, in a number of cases calculation methods similar to those noted above are used. However, the condition $kd < 1$ limits the application of such an approach with energies no less than 100 Mev/nucleon when the microvolume measures 0.1-1.0 μμ, i.e., it makes it practically suitable only for protons with energies in hundreds of mega-electron volts.

This approach is also applicable for heavy ions with energy up to several mega-electron volts per nucleon if DNA is considered as the biological object [10] for which $d \sim 2\text{-}3.10^{-3}$ μμ. The use of $f_1(z)$ calculated in this way by many authors along with G(z) obtained from radiobiological experiments produced good agreement between calculated and experimental data on the inactivation of E. coli.

A different approximate calculation of the energy transferred by δ-electrons was used in work [71] with simulation of the radiobiological effect from a dose of multi-charge ions. The authors calculated the radial distribution of energy transferred by δ-electrons (RDET) in the ion track and then averaged the radiobiological effect through a cross section of the track on the basis of dose-effect dependence obtained in γ-radiation. This RDET has the form $\sim 1\rho^2$, which in this case is equivalent to using $f_1(z) \sim 1/z^2$. It follows that

calculation of the effect of energy transferred by δ-electrons essentially changes the form of $f_1(z)$ in comparison with consideration of primary particles only. Such an approach is applicable when $d > 1$ and the microvolume is small in comparison with ρ_{max}, which, as seen from Fig. 40, is observed for ions with charge $z \geq 6$ and for many biological objects used in radiobiological experiments in existing multi-charge ion accelerators.

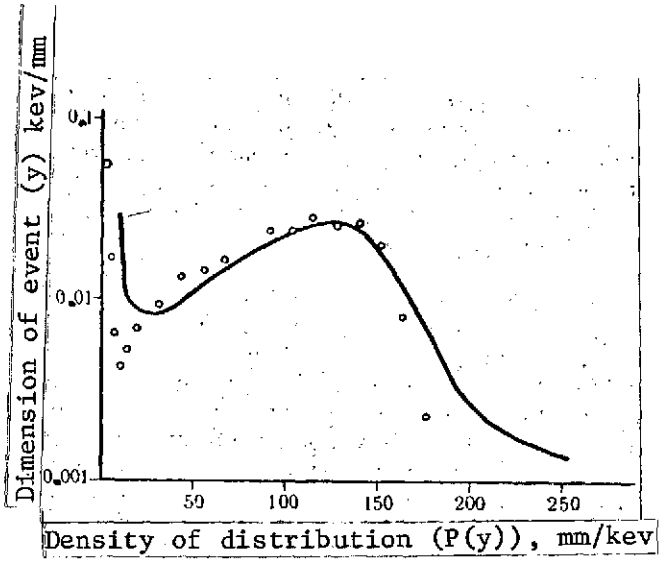


Figure 43. Density of distribution of dimensions of events P(y) in a spherical area 0.5 μμ in diameter.

Experimental curve for d-particles, Am^{241}, obtained in work [60], points calculated by the Monte Carlo method taking into account energy transferred by δ-electrons for α-particles with energy of 4 Mev (2000 histories).

For the most complete calculation of all causes of fluctuation in energy transferred to the microvolume it is also necessary to model the trajectories of both the initial charged particles and primary, secondary, etc. electrons by the Monte Carlo method on electronic computers. As an example of direct modeling of the trajectory of electrons we can use work [49], in which the density of distribution of energy in a spherical volume was calculated both for isotropic monoenergetic electron sources, evenly distributed over the surface of the sphere, and for sources evenly distributed within the volume and in the environment. The calculation technique in which the Monte Carlo method was used to model the trajectory of δ-electrons is quite tedious and requires considerable expenditure of time on an electronic computer. However, as follows from the above analysis of calculation methods, only by using such a technique was it possible to obtain correct densities of distribution of $f_1(z)$, appli-

cable to objects studied in radiobiology for various kinds of radiation in the area of dE/dx where the factor of quality is greater than one.

Based on the above outlined requirements, we devised a calculation technique and program on an electronic computer to determine $f_1(z)$ in spherical microvolumes radiated with protons and multi-charge ions, taking into account the trajectories of δ-electrons. The application of this program to the task considered in work [149] showed good coincidence of results (Fig. 42). Calculations of the distribution of $F_1(z) = f_1(z)dz$ for protons with energy of 4 and 9 Mev and $d = 1$ μμ (see Fig. 41) made according to this program show that calculating the effect of δ-electrons markedly reduces the average absorbed energy transferred to the microvolume by one charged particle in comparison with work [64]. Calculations for α-particles with energy of 4 Mev and a microvolume of 0.5 μμ give the density of distribution of absorbed energy (Fig. 43) containing part $1/z^2$ type, caused by δ-electrons, and part triangular kind, formed by α-particles. Fig. 43 also shows the experimental results of the determination of density of distribution of absorbed energy using a wall-less proportional counter with dimension equivalent to $d = 0.5$ μμ obtained for α-particles of Am^{241} in work [60]. This agreement between calculation and experiment can be considered sufficiently good. /176

As can be seen, the distributions of $f_1(z)$ are essentially different in cases when the transfer of energy by δ-electrons is taken into consideration or is not. However, the effect of this difference on the radiobiological influence, as follows from formula (1), depends on the specific kind of G(z). If there is quite accurate information in relation to f(z, D) or techniques making it possible to obtain such, the kind of G(z), determined by the development of radiation damage in the biological object, has at present no accurate quantitative description; therefore, G(z) is chosen from model representations.

One of the simplest kinds of G(z) is that in stages which is essentially contained in the multi-impact theory if it is considered that the same amount of energy is transferred in each hit; it is also used by many authors in com-

bination with quite accurate expressions for f(z, D), for example, in the work of Hug and Kellerer [64]. In analyzing processes of post-radiation recovery in work [26] G(z) in the form $1-q^{\alpha z}$, $1-\left(\frac{q}{\alpha z}\right)^{\alpha z}$ and $1-(q\alpha z)^{\alpha z}$ were discussed where α is the constant taking into consideration dimension. Dependence $G(z)=1-q^{\alpha z}=1-\exp[(\alpha z \ln q)]$ satisfies the independent recovery of primary injuries with probability not depending on radiation characteristics. This approach is used in work [16]. In this way, modifications of G(z) in the form $1 - q^{az}$ with $z < z_p$ are considered as well as the step with $z = z_p$ where z_p is a certain threshold value of energy.

By trial and error of value q many authors were able to obtain coincidence between calculated dose-effect curves and those obtained experimentally in roentgen and γ-radiation. In a number of cases, in order to obtain coincidence with experiments, models were complicated by calculation of the degree of structure of sensitive volumes of the biological objects [10, 18], the simplest example of which is the multi-target theory.

However, the above noted G(z) models were not verified with regard to a description of the experimentally-observed dependence of the cross section of radiation injury on dE/dx. An attempt to obtain this dependence was undertaken in work [20] in combination with a calculation of $f_1(z)$, assuming that with each hit energy transferred by the charged particle is distributed according to Poisson law with average value proportional to dE/dx. With the use of G(z) in step form a reduction in survival with increased dE/dx could be obtained only in small doses; with large doses the opposite effect was produced [18]. With the use of G(z) in the form $1 - q^{az}$ in combination with the same kind of $f_1(z)$ there is obtained a dependence of the cross section of radiation injury in the form $\sigma=\sigma_0(1-e^{-k\cdot\frac{dE}{dx}})$ [20]. Such dependence qualitatively coincides with experiments on a number of biological objects for which the survival curve bears an exponential character even with γ-radiation. More accurate coincidence was obtained in work [71] where dependence σ(dE/dx) was calculated on the basis of RDET information and as G(z) was used the exponential curve of survival obtained with γ-radiation, corresponding to the model

of independent recovery with constant probability.

However, the method suggested by the authors of [71] is used only in those cases when $f_1(z) \sim 1/z^2$ which occurs with rather high energies of heavy ions and small dimensions of biological objects. In other cases frequently /177
encountered in practice similar calculations require the use of a more accurate kind of function $f_1(z)$, making it possible to carry out the program we devised.

A more complex task is modeling the dependence of the cross section of radiation injury on dE/dx for those biological objects characterized by increased identity in the range of dE/dx between 10 and 100 kev/μμ. The analytic approximation of σ(dE/dx), suggested for this case in work [90], has the form $\sigma = \sigma_0\left(1 - e^{-\alpha\frac{dE}{dx} - \beta\left(\frac{dE}{dx}\right)^2}\right)$. The model of probability of realization, of fixing primary injury, meeting such approximation was suggested in work [20] in the form of dependence $G(z) = 1 - \left(\frac{c}{a + b\frac{dE}{dx} + c}\right)$, which with $c > a + \beta\frac{dE}{dx}$

$$G(z) \approx z\frac{a + b\frac{dE}{dx}}{a + b\frac{dE}{dx} + c}.$$

In this work the opinion is expressed that a radiobiological effect appears when primary injury is fixed in only one of the injured sections of sensitivity of the volume of the biological object; participating in fixing primary injury are both chemical agents continually present in the environment and those which are short-lived products of the interaction between radiations and the environment. In this case the concentration of such products in the charged particle track, i.e. close to the injured sections, can be recognized as proportional to dE/dx and the probability of fixation of primary injuries of sections linearly dependent on dE/dx which provides quadratic dependence of the cross section on $\frac{dE}{dx}$ in the range from 10-100 kev/μμ also reaches a plateau with large values.

An approach to modeling G(z), close to that outlined above, making it possible to obtain an increase of RBE with increased dE/dx by calculation on the basis of microdosimetric information is contained in work [72]. Studying the dependence of RBE on dosage, the authors used in their calculations the quadratic dependence of the probability of "elementary injuries" on energy z absorbed by the microvolume. As possible mechanisms meeting such dependence, the authors name injury to two sections, leading to the development of "elementary injury," or interaction of any other products formed by radiation in the biological object.

It is obvious that the mechanism of radiation injury is subject to serious study; however, the dependence of G(z) could be modelled at the present time on the basis of microdosimetric information. We are considering obtaining the distribution of $f_1(z)$, taking into account the trajectory of δ-electrons, by the Monte Carlo method according to the devised electronic computer program. As an approximation of G(z), it is, evidently, necessary to select a dependence reflecting the interaction of the two products of the effect of radiation on the biological object; analysis of radiobiological information must give the values of its coefficients.

N75 14426

Methodological Questions of Creating Tissue-Equivalent Phantoms

/178

To evaluate the radiation danger in outer space it is necessary to have information on the characteristics of radiation in the body of the cosmonaut. This information is obtained by calculation or experimental methods with the use of phantoms modelling the body of the cosmonaut. In cases when the radiation effect on the cosmonaut is caused by strongly diffused or absorbed radiation, as well as in the presence of a significant portion of secondary radiation, it is desirable to reproduce as accurately as possible the actual shape of the biological object. The desire to conduct phantom studies in real anatomical geometry, when the actual portion of diffusing and secondary radiation

is provided, necessitates the creation of phantom-dummies conforming as closely as possible to the shape and geometric dimensions of all parts of the human body.

In creating phantoms it is necessary to have certain average data on the height of the man, his weight, to know average chemical composition of the body and that of various organs. In works [77, 37, 82] there is information on dimensions of organs, their chemical composition and that of the body. Work [45] gives body measurements. A combination of such statistically averaged information gives a representation of a certain "standard" man.

Tables 41-44 give certain data on this "standard" man.

TABLE 41

BASIC PARAMETERS OF A "STANDARD" MAN

Parameter	Literature source		
	[82]	[77]	[37]
Body weight, kg	70	70	70
Height, cm	170	--	--
Surface of the body, m^2	1.8	1.5 – 2.0	--
Water content, kg	40 – 46	50	43

In selecting physical parameters and chemical composition for a "standard" man, preference must be given to work [37], which contains the recommendations of the International Commission on Radiological Protection.

In creating a phantom, determining parameters are primarily weight and height. If there are no differing opinions with regard to weight and that of the "standard" man is considered to be 70 kg, the height of this "standard" man and the geometrical dimensions of individual sections of the body are assumed differently in different works. Therefore, we studied carefully European anthropological data for males, as the result of which the average

height of the man was assumed to be 170 cm with weight of 70 kg. On the basis of a study of anthropological data, geometrical dimensions were also obtained for various cross sections of the body of the "standard" man, without knowledge of which it is impossible to create a phantom of a "standard" man. The location of the cross sections is given in Fig. 44.

TABLE 42

/179

AVERAGED CHEMICAL COMPOSITION OF THE BODY OF A "STANDARD" MAN (IN PERCENT OF BODY WEIGHT) AND WEIGHT OF COMPONENTS

Element	Literature source			Mass, g
	[82]	[77]	[37]	
Oxygen	65	65	65	45,500
Carbon	18	18	18	12,600
Hydrogen	10	10	10	7,000
Nitrogen	3	3	3	2,100
Calcium	1.5	1.5	1.5	1,050
Phosphorous	1.0	1.0	1.0	1,050
Sulfur	0.25	0.25	0.25	174
Potassium	0.35	0.2	0.2	140
Chlorine	0.15	0.15	0.15	105
Sodium	0.15	0.15	0.15	105
Magnesium	0.005	0.05	0.05	35
Iron	0.004	0.006	0.0057	4.2
Zinc	--	--	0.0033	2.32
Rubidium	--	--	0.0017	1.2
Strontium	--	--	0.0002	0.14
Copper	0.002	0.002	0.0014	0.098
Total ...			100%	70,000

As in the usual case the cosmonaut can be exposed to the radiation effect of protons, neutrons and γ-rays, material for the phantom must be tissue-equivalent with regard to these kinds of radiation. With regard to γ-radiation, tissue equivalency of the material is practically independent of its composition and is determined on the basis of effective atomic number z_{eff}. Neutrons transmit their energy to tissue as the result of nuclear interactions, elastic diffusion in nuclei of tissue, slowing down the neutrons before thermal energies if dimensions of the medium make it possible, and subsequent cap-

TABLE 43

CHEMICAL COMPOSITION OF MUSCLES AND BONES OF "STANDARD" MAN (IN PERCENT OF THEIR WEIGHT)

Element	Muscle tissue [65]	Bone (thigh) [65]
Oxygen	72.9	41.0
Carbon	12.3	27.8
Hydrogen	10.2	6.4
Nitrogen	3.5	2.7
Calcium	0.007	14.7
Phosphorous	0.02	7.0
Sulfur	0.5	0.2
Potassium	0.3	--
Sodium	0.08	--
Magnesium	0.02	0.2
Total...	100	100

TABLE 44

CHARACTERISTICS OF VARIOUS CROSS SECTIONS OF THE HUMAN BODY

Element of measurement	Location in Fig. 44	Length of perimeter, mm
Pelvis	1	1,000
Abdomen	2	790
Chest	3	1,000
Head	4	580
Biceps	5	305
Lower part of arm	6	250
Thigh	7	495
Knee	8	390
Calf	9	375

ture of thermal neutrons primarily by nuclei of hydrogen [reaction of H' (n, γ)] and nitrogen [(reaction of N^{14}(n, p) C^{14}]. Evaluations show that tissue-equivalency of the material to neutrons is provided by the weight content, primarily of hydrogen and nitrogen. /180

Basic processes of energy transfer of protons to tissue are ionization losses dE/dx and nuclear interactions. As dE/dx for hydrogen are more than double the ionization losses of protons in nuclei of the carbon, nitrogen and oxygen group, the content of hydrogen is again essential for providing tissue-equivalency of the material. In relation to nuclear interactions of nucleons, literature data indicate that cross sections of interaction, energy spectra and the production of secondary particles for nuclei of the carbon, nitrogen and oxygen group undergo weak changes [91]; therefore, change in the content of the two basic tissue elements remaining unfixed — carbon and oxygen — will not markedly disturb the tissue-equivalency of the material. However, when oxygen is completely replaced in tissue by carbon the macroscopic cross section of the interaction between nucleons and tissue changes approximately 10%.

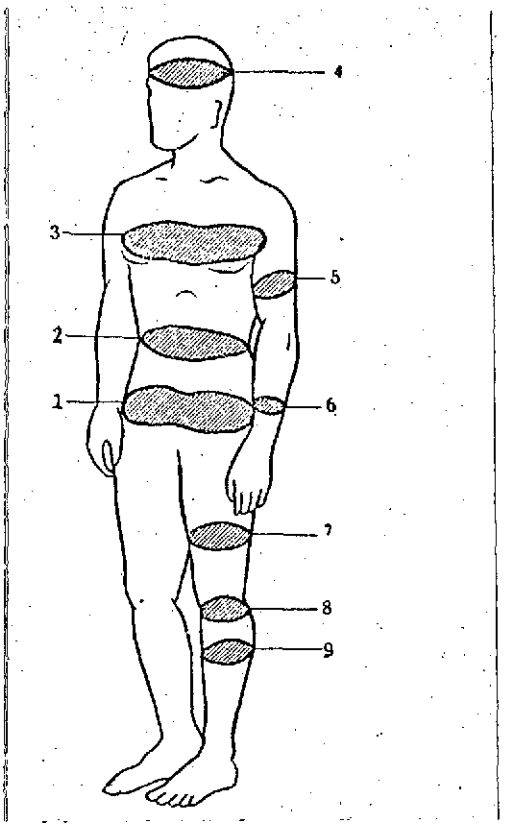


Figure 44. Characteristics of various cross sections of the human body.

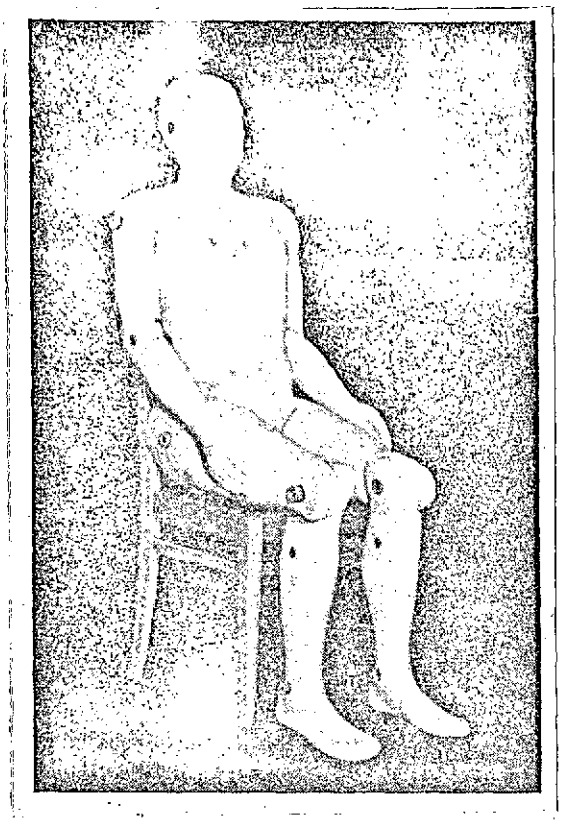


Figure 45. Phantom made of tissue-equivalent material.

In connection with this, in selecting a tissue-equivalent substance most preferable are materials in which the carbon and oxygen content is close to that of tissue. /181

Thus, these evaluations show that material, tissue-equivalent in relation to γ-radiation, protons and neutrons, must include 10% hydrogen and 3.5% nitrogen with $z_{eff} = 7.42$.

The composition of tissue can be reproduced exactly at room temperature by a mixture of solutions. However, a tissue-equivalent substance in the form of a solution or a mixture of solutions cannot be used to create a human phan-

tom for studies in outer space. Therefore, at present we are devising a plastic which, as evaluations have shown, is tissue-equivalent in relation to γ-radiation, neutrons and protons with about 5% accuracy.

A phantom intended for research on the problem of the radiation safety of space flights, as well as radiation safety in ground nuclear equipment used in radiation therapy, must be heterogeneous. It is most essential to specify a skeleton and lung tissue in the dummy as being significantly different in specific weight from tissue (1 g/cm^3)(and the skeleton differing in composition as well), they strongly alter the characteristics of radiation within the dummy. In connection with this, there was the problem of devising forms for creating a dummy, taking into account the inclusion of a skeleton and material simulating lung tissue.

As is known, lungs weigh about 1 kg with density of 0.3 g/cm^3 and bone weighs 7 kg with density of 1.3 g/cm^3 [37]; the volume of a dummy weighing 70 kg with average density of 1 g/cm^3 is about 70 l. To create a phantom first a plaster model of a "standard" man was made with parameters: height 170 cm, volume 70 l. Body parts of this model of a "standard" man had the dimensions indicated in Table 41. The model was used to make plaster forms for casting dummies out of tissue-equivalent material. A phantom of tissue-equivalent material was cast in these forms (Fig. 45). The dummy is the first stage in working out the creation of phantoms with movable limbs. Certain difficulties at this stage of the work were connected with designing limbs with the necessary condition of preserving cross sectional dimensions and length of parts of the "standard" human body.

The second step in devising a method of making phantoms was the creation of metallic-aluminized forms. The difficulty here was that for each part of the body it was necessary to make a form consisting of only two parts without any negative angles.

C-4

Thus, on the basis of analysis and generalization of literature data, the composition of tissue-equivalent plastic was justified, parameters of a "standard" man were determined, plaster and metal forms were created for casting dummies and an experimental model was produced from tissue equivalent material.

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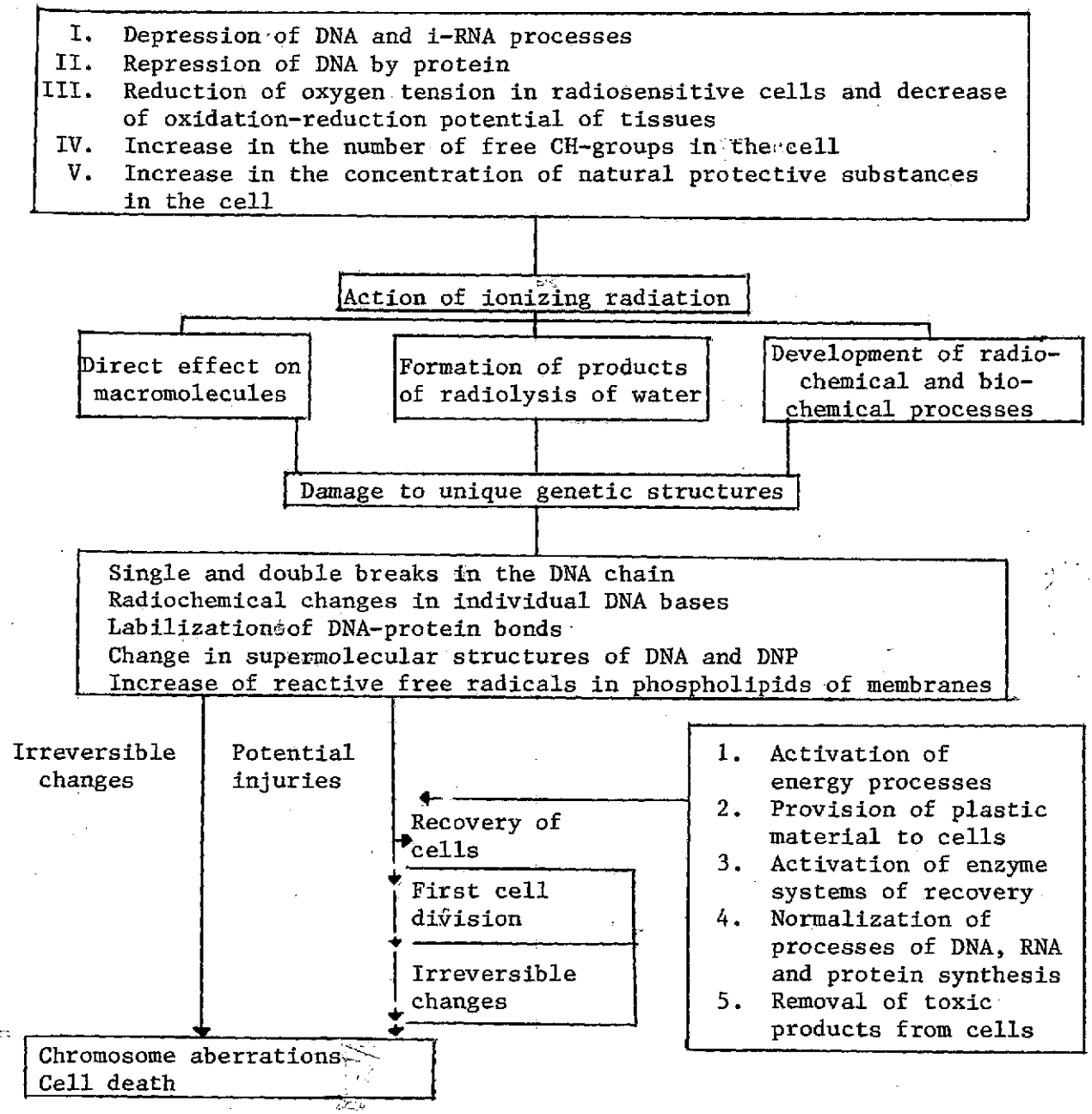
PHARMACOCHEMICAL PROTECTION AGAINST IONIZING RADIATION /186

Pathogenetic Validation of the Use of Biological Protective Agents and Early Treatment in Cases of Radiation Injury Simulating Radiation Effects under Space Flight Conditions

In considering a radiation safety system for space flights, the various measures to protect man against radiation can include the use not only of means of physical protection, but also drug prophylaxis.

At the present time a great deal of experimental material has been accumulated on the prevention and treatment of radiation injuries [45, 46]. Anti-radiation effectiveness has been established for sulfur- and nitrogen-containing substances, auxins, cyanides, polynucleotides, mucopolysaccharides, lipopolysaccharides, aminosaccharides, synthetic polymers, vitamins, hormones, amino acids and other compounds which, according to our classification and /187 data on the mechanism of their action and characteristics of the most optimum administration, can be divided into two basic groups — biological and chemical protective agents [28, 36].

Current analysis of ways of preventing radiation injuries, as well as information on mechanisms of the action of agents able to increase post-radiation recovery processes, have made it possible to devise a working plan on


I. Depression of DNA and i-RNA processes
II. Repression of DNA by protein
III. Reduction of oxygen tension in radiosensitive cells and decrease of oxidation-reduction potential of tissues
IV. Increase in the number of free CH-groups in the cell
V. Increase in the concentration of natural protective substances in the cell
Action of ionizing radiation
Direct effect on macromolecules
Formation of products of radiolysis of water
Development of radiochemical and biochemical processes
Damage to unique genetic structures
Single and double breaks in the DNA chain
Radiochemical changes in individual DNA bases
Labilization of DNA-protein bonds
Change in supermolecular structures of DNA and DNP
Increase of reactive free radicals in phospholipids of membranes
Irreversible changes
Potential injuries
Recovery of cells
First cell division
Irreversible changes
Chromosome aberrations
Cell death
1. Activation of energy processes
2. Provision of plastic material to cells
3. Activation of enzyme systems of recovery
4. Normalization of processes of DNA, RNA and protein synthesis
5. Removal of toxic products from cells

which to base studies of pharmacochemical protection against the effect of cosmic rays (see diagram). In the very first stages of this work, which was /188 begun in 1968, it was decided in conduct studies in the "Interkosmos" program

to investigate the effect and validate the most optimum use of biological protective agents able to raise the natural radioresistance and general resistance of the body, as well as means of early pathogenetic treatment of radiation sickness.

As is known, works in previous years have determined the basic characteristics of biological protective agents — primarily their ability to have an anti-radiation effect when administered several days or a week before ionizing radiation not causing absolutely lethal radiation damage (i.e. LD_{20}-LD_{80}) and with the administration of the drugs in doses not, as a rule, causing pronounced toxic reactions.

In conducting works in the "Interkosmos" program, principal attention was given to studying the effect of the following groups of compounds: 1) amino acids and vitamins; 2) hormonal preparations; 3) polysaccharides of bacterial origin. Studies with amino acids and vitamins made it possible to work out and study comprehensively the characteristics of the effect of amitetravit, which is a combination of vitamins B_1, B_6, P and C and amino acids — tryptophan and histidine.

An additional verification of the productivity of studies in this direction were the works of Czechoslovakian scientists who established the pronounced prophylactic effect of 5-azacytidin administered 24-72 hours before radiation. This effect consists of the ability of this compound to increase reliably the survival of radiated mice and affect favorably the hematopoietic system [136].

The effect of hormonal preparations as a means of prophylaxis was studied by Polish and Russian scientists. Tests on small laboratory animals with brief and prolonged radiation showed the ability of anabolic steroids to activate significantly protein synthesis and the synthesis of DNA, to have an anti-radiation effect [79-80, 91, 109-111]. The administration of methandrostenolone to guinea pigs in doses of 1 mg/kg 21 days before radiation in doses of

LD_{60}-LD_{80} and 18 days after radiation caused a reliable protective effect (20-40% increase of survival). The reduced dose factor (RDF) was 1.6 according to survival. Peroral prescription of methandrostenolone in doses of 1 mg/kg 20 days before radiation and 20 days after reduces disturbances in the protein composition of blood serum; this appeared as a lesser reduction in the content of albumins, γ-globulins and properdin. The ability of methandrostenolone to reduce disturbances in the respiratory function of tissues and their content of radiotoxins was also shown.

Soviet researchers [24, 39] in an analogous schedule of methandrostenolone use revealed its favorable effect on the blood system, especially on erythropoiesis.

Special interest is given works showing the erythropoietic effect of methandrostenolone in prolonged radiation. It was seen in the reliably higher number of erythroblastic elements in bone marrow and the spleen in comparison with control radiated animals. In addition, the therapeutic effect of methandrostenolone under such conditions of administration appeared in the early large content of highly-resistant erythrocytes and the significantly lower /189
content of poorly-resistant red blood cells, as well as in its ability to normalize the synthesis of iron after radiation and to stimulate the formation of hemoglobin.

On the basis of data of Polish scientists, the impression is given that anabolic steroids in prophylactic and protective-therapeutic use in cases of acute radiation sickness are able to prevent catabolic symptoms in the radiated organism and lead to certain normalization of DNA and RNA synthesis. In addition, the works of Polish scientists have recently shown the favorable effect of methandrostenolone on post-radiation disturbances in protein synthesis (discovered by the metabolism of C^{14}-leucine). It was shown that in normal animals 20-day administration of methandrostenolone causes a reliable increase in the inclusion of C^{14}-leucine in the protein of homogenates and all studied subcellular fractions of the liver. This inclusion was especially high (over

200% in relation to the control) in the mitochondrial fraction.

Studies of enzymes of the respiratory cycle established that methandrostenolone increases the activity of dehydrogenase in succinic acid 32% and cytochromoxidase 22%. It is assumed that methandrostenolone basically stimulates the synthesis of mitochondrial proteins and enzymes found there, and actively interferes in enzyme, energy and oxidation processes in cells, playing an important role in the mechanisms of its anti-radiation effect [111]. The significance of this phenomenon was also revealed in the works of Czechoslovakian scientists [137]. It was established that with the use of hormonal agents the radioresistance of the body is altered by change in the activity of, for example, glucose-6-phosphate dehydrogenase. A similar effect was recorded in tests on mice with the use of dehydroepiandrosterone in doses of 50 mg/kg administered 16 hours before radiation. In such cases dehydroepiandrosterone gives a more pronounced radioprotective effect [124].

All these studies by scientists of Poland, the USSR and Czechoslovakia indicate, in our opinion, the validity of using anabolic steroids in cases of radiation injury. Especially encouraging in this respect are the prospects for possible use of anabolic steroids during prolonged radiation at low rates.

Developing research to study the effect of hormonal preparations, scientists of socialist countries also obtained interesting material regarding the significance of the state of the adrenocortical system in forming increased radiosensitivity. It was shown that animals in the resistant stage of a general adaptation syndrome (which can be revealed by testing the 11-oxycorticosterone content in the blood) show increased radioresistance. Such radioresistance can be created, in particular, by the administration of ACTH in doses of 10-15 units/kg 3 hours before radiation. When mice are radiated in doses of LD_{60}-LD_{80}, the use of ACTH causes 30-40% increased survival of the animals. Noteworthy is the fact that the prophylactic effect of ACTH completely disappeared when the radiation dose was increased from sublethal to absolutely lethal [37].

Besides analysis of the adrenocortical system, studies of biogenic amines in peripheral blood are also important for determining the state of natural radioresistance. A clear dependence was established between the severity of clinical symptoms of radiation sickness in animals (dogs and monkeys) radiated in doses of LD_{60}-LD_{100} and the initial level of serotonin in peripheral blood. /190 In animals having the highest normal content of serotonin in the blood before radiation, the clinical symptoms of radiation sickness after radiation were weak and, as a rule, these animals survived (at LD_{80}). On the other hand, animals having the lowest amount of serotonin in the blood (in the normal physiological state) before radiation typically underwent a more severe course of radiation sickness after radiation and basically all died [35].

The third important direction in experiments with biological protective agents was a study of polysaccharides of bacterial and plant origin. Primary attention was given to the effect of such means of preventing radiation sickness as lipopolysaccharide extracted from E. coli bacteria, zymosan — a polysaccharide extracted from yeast cells, prodigiosin — a polysaccharide obtained from Bacillus prodigiosus, caraginin extracted from algae and dextran.

Works conducted by Hungarian scientists (Berton) with lipopolysaccharides extracted from E. coli established that this preparation administered 1-2 days before radiation (LD_{70}-LD_{95}) in doses of 100-500 mg/kg intramuscularly in tests on Wistar hybrid rats increases survival of the animals 50-80%, improves the state of the blood system and eases the clinical course of radiation sickness. In addition, it was established that the prophylactic administration of lipopolysaccharide extracted from E. coli can help protect stem hematopoietic elements and prevent disturbances in the genetic apparatus of bone marrow cells.

There was a lesser effect from the prophylactic administration of zymosan. Given to mice 24 hours before radiation, this increases survival of animals radiated in doses of LD_{60}-LD_{85} 25-28%. This effect appeared with administration of the drug in doses of 20-125 mg/kg [55].

The use of prodigiosin produced better results. Its positive effect was detected both in prophylactic use 24 hours before radiation and in therapeutic use in the first 3 hours after radiation injury [12, 57]. These variants of the use of prodigiosin increased survival of radiated mice and guinea pigs 50%. Deserving of special attention are data indicating the ability of prodigiosin to produce an anti-radiation effect (20% increase of survival) with prolonged radiation.

A high prophylactic effect with brief radiation was detected from the use of caraginin. In tests on mice its positive effect (LD_{100}) appeared in the 60-70% increased survival of the animals [58]. An analogous anti-radiation effect was found by Hungarian scientists using endotoxin extracted from S. Thiphi on mice after radiation (20 minutes) [132].

The use of dextran with a molecular weight of 3.18×10^5 and 1.37×10^5 increased the survival of protected mice 26-63%. The highest protective effect was found in animals protected by dextran with a molecular weight of 3.18×10^5 in doses of 1500 mg/kg. The use of native dextran with a molecular weight of $1.5 < 10^5$ and hydrolyzed dextran with a molecular weight of 47.5 thous. was practically without result. The use of dextran in case of prolonged radiation had no effect [49].

These studies, therefore, indicate prospects for using polysaccharides /191
of various origin as anti-radiation means. Of all the samples of polysaccharides studied, greatest attention is, evidently, due prodigiosin used with brief and prolonged radiation. Definite prospects are also noted in relation to caraginin and other polysaccharides as anti-radiation agents. However, there are still insufficient studies to make a more definite judgment of the advisability of their practical application.

These anti-radiation properties of polysaccharides spurred special research to study the mechanism of their effect on the hematopoietic system. The transfusion method of Till and McCulloch was selected for comparative

evaluation of the protective effect on the hematopoietic system. These studies showed that most effective in relation to protecting the colony-forming elements of bone marrow are caraginin and prodigiosin [42].

In discussing the mechanism of the effect of all studied means of biological protection able to raise the natural resistance of the body, first of all, we must take into consideration the fact that natural radiosensitivity is a genetically-dependent property. This also refers equally to the frequency of chromosome and gene mutations. It is known, for example, that radioresistant varieties of mice are at the same time more resistant to a variety of external effects, even infectious diseases and various toxic factors. This indicates the presence of general protective-restorative systems protecting vital structures from damage, regardless of which factors cause it.

The existence of these systems is also indicated by studies of the mechanism of acclimatization to hypoxia, muscular training and hardening to cold. Noteworthy is the fact that acclimatization to hypoxia raises the resistance of the body not only to the effect of acute hypoxia and radiation, but also to infections and various kinds of intoxications.

On the basis of accumulated data, in our opinion, it can be assumed that the formation of the natural resistance of the body with the help of biological protective agents is connected with their ability to increase the activity of the adrenocortical system, the base of hematopoienia and the potential of hematopoietic cells for proliferation, to stimulate the function of the reticulo-endothelial system and immunological reactivity. It is also necessary to point out that under the effect of biological protective agents, changes can arise not only in cellular and organism metabolism, able to create an elevated natural radioresistance and modify processes of post-radiation recovery, but also in corresponding shifts at the molecular level, preventing disturbances in the DNA molecule, protein synthesis, etc. These effects can occur because biological protective agents injected into the body before radiation accelerate processes of protein synthesis [32, 51, 109-110]. This mechanism is ex-

tremely important, as it is known that although at the moment of radiation proteins are being synthesized in sufficient quantity, corresponding genes can be screened by histones. Under such conditions DNA damages can be reduced several times and breaks in DNA chains more than twice. In discussing the possibility of radiation degradation of DNA contained in DNP it was shown /192 that the degree of protection by protein can appear in the 2-3-fold reduction in the number of individual breaks [62, 64].

We must also not fail to take into consideration other aspects of the effect of biological protective agents at the molecular level, connected with stimulation of protein synthesis. It was established that DNA contained in the nucleoproteid complex is in a more densely packed state and reduction of the volume of the target could lead to increased resistance of DNA in the complex in comparison with free DNA.

Special significance in the mechanism of the effect of biological protective agents must, evidently, be given to changes in the adenyl cyclase system and the accumulation of cyclic 3'-5'-adenosine monophosphate in cells of radiosensitive organs which is able to increase the amount of nicotinamide adenine dinucleotide in cells, to activate enzyme systems of the restoration of genetic structures and to ensure biosynthesis of nucleic acids. In the final analysis, all these biochemical shifts could play an important role in regenerative processes and metabolism regulation in cells injured by ionizing radiation.

These reports find verification in materials showing that ACTH, anabolic steroids and several other hormonal agents can activate the cellular cycle and increase the content of cyclic 3'-5'-adenosine monophosphate in cells [65, 78, 121].

Lipopolysaccharides of bacterial origin also act, evidently, by an analogous mechanism. Entering into a mutual relation with the effector cell, basically receptors of the adenyl cyclase system connected with the cell membrane,

they activate the adenyl cyclase system, whose function is transforming ATP into cyclic 3'-5'-monophosphate. A verification of this mechanism by biological protective agents can be the prophylactic effect of cyclic 3'-5'-monophosphate itself [96-99]. Also important is establishment of the pronounced anti-radiation effect from the use of ATP [36, 41, 105]. The significance of the activation of the adenyl cyclase system and the pentose cycle in the mechanism of biological protective agents was also found recently in studying the effect of glycine and γ-oxybutyric acid which were used in works by scientists of Romania and Czechoslovakia [74, 76-77, 86, 123, 125-126].

All these biochemical shifts should also be taken into consideration in discussing the positive effect of biological protective agents on changes in the hematopoietic system, in particular, in analyzing the protective effect of polysaccharides on stem hematopoietic elements [60]. As is known, according to current opinions, this plays an important role in restorative processes in the hematopoietic system and immunological reactivity. These cells in the proliferating pool, able to reproduce similar elements for an unlimited time, can cause the expansion of regenerative cellular colonies which, as differentiating stimuli appear, are transformed into erythro-, myelo- and immunoblasts.

A verification of the correctness of this theory is the recent work of USSR and Hungarian scientists searching for ways to control the transformation of stem hematopoietic cells. It was shown that the use of certain polysaccharides and endotoxins of bacterial origin could have a controlling effect on mother cells, favoring differentiation of stem cells which are induced by the direct or mediated effect of ionizing radiation [42, 57, 132]. /193

As has been established, biological protective agents are able to increase the amount of properdin in the blood of radiated animals and increase immunological reactivity [1, 25, 32]. An important addition to this information on the mechanism of biological protective agents is made by studies of Hungarian and USSR scientists who found that lipopolysaccharides of bacterial

origin and other agents raising natural resistance and radioresistance, for example, a synthetic double-helix complex of polyinositol and cytidinic acids and Newcastle Disease virus, are inducers of interferon [43, 128]. Therefore, the theory of the polypotency of stem cells can be used as the basis to explain the protection of hematopoiesis and the increased anti-infection resistance with the use of biological protective agents and thus determine possible new pathways of search for anti-radiation drugs.

In discussing further prospects for using biological protective agents during space flights, especially noteworthy is the increased resistance of the body under the effect of such agents not only to radiation and extreme space flight factors of a non-radiation nature [14], but also to a number of pathogenetic effects, even resistance to traumas. In this connection it is especially necessary to dwell on the studies of Czechoslovakian scientists who used experiments with hormonal preparations (19-nor-testosterone phenylpropionate) to show the close connection between their anti-radiation activity and the effect on tolerance of mechanical traumatization, burns, starvation, etc. [90].

Studies in this direction regarding the prophylaxis of radiation sickness indicated the advisability of further expansion of experiments with biological protective agents in relation to the aims of space medicine. However, before such studies were undertaken, taking into account the vast experience of experimental investigation of chemical protective agents and their high anti-radiation effectiveness in tests on mammals radiated briefly at a high rate [45-46], it seemed necessary to conduct special works with this group of prophylactic drugs for comparison with biological protective agents in the "Interkosmos" program. An important prerequisite to studies of chemical protective agents was the information that many of these, for example, β-mercaptoethylamine (mercamine hydrochloride, ascorbinate, bitartrate), β-mercaptoethylamine disulfide (cystamine), β-mercaptopropylamine hydrochloride (propamine), 5-methoxytryptamine (mexamine), S-β-amino-ethylisothiourea (AET), etc., are already authorized for use in medical practice. In addition, attention is

given to experimental research on various kinds of animals which showed the ability of chemical radioprotectors to produce an anti-radiation effect with minimum absolutely lethal radiation injuries when administered immediately before radiation.

Our studies verified known data that the basic characteristics of chemical protective agents are their ability to prevent changes, primarily in radiosensitive organs and tissues, to preserve in them a certain portion of cellular elements and, what is especially important, colony-forming elements of bone marrow and the epithelium of intestinal mucosa. With the use of the most effective drugs necrobiotic processes and the number of cells with chromosome aberrations are reduced in hematopoietic organs and the intestine, there is more rapid recovery of mitotic activity and a certain correlation is observed between the severity of these processes in the first hours after radiation and survival of the animals [30, 31, 67, 81, 107, 133-135]. /194

At the same time it was clearly shown that the use of these agents is accompanied by a number of side toxic reactions which severely limit the possible aspects of their use during space flight. This prompted us to conduct a series of works to reduce the toxicity of chemical radioprotectors and to investigate new low-toxic anti-radiation drugs. Greatest successes in this area have been reached in recent years as the result of the development of radio-protective formulas. Much success has been attained by Bulgarian scientists who suggested formulas with a marked anti-radiation effect. In tests on mice and rats radiated with x-rays (LD_{90}-LD_{100}), the use of a mixture of radioprotectors consisting of ATP, AET, serotonin or ATP, serotonin and S-2-benzimidazolyl mercaptopropio-N-lp-tolyl-l-hydrazidine caused a 70-95% increase in survival of the animals. It is important to note that in these combinations minimum doses of the radio-protectors were used which in isolated use did not cause pronounced toxic reactions [26, 69, 105-106, 112-113].

A high protective effect with the combined use of chemical and biological protective agents was obtained by Polish scientists. According to the data of

Panek [109-110], with the combined prophylactic use of dianabol, DNA, hydro-oxybutyric acid and cystamine in rats radiated with x-rays, RDF was 2.7. An increased protective effect was also established, especially on the hemato-poietic system, from the combined administration of dianabol and hydro-oxy-butyric acid, dinitryl succinic acid or DNA.

Also noteworthy is the series of works of Czechoslovakian scientists validating the combined use of chemical radio-protectors and biological protective agents. In particular, on the basis of these studies, the high anti-radiation effect from the combined use of plant urease as a biological protective agent and AET was shown [87-88]. A pronounced anti-radiation effect was established for urease alone. It appeared not only in the 20-50% increased survival of radiated animals, but also in the prevention of necrobiotic changes in intestinal mucosa and a reduction in the level of chromosome aberrations in bone marrow and epithelium of intestinal mucosa.

An important observation is that of Czechoslovakian scientists regarding the ability of urease to give a positive effect even when the interval between the administration of the drug and radiation was several days. With the combined use of urease and AET the above indicated anti-radiation effects were considerably increased. Survival of radiated mice increased (at LD_{80}-LD_{100}) to 60-70%. In addition, such administration of urease and AET makes it possible to reduce the radio-protective dose of the latter extremely toxic drug. /195

Numerous studies have been conducted by scientists of Bulgaria, the USSR and Czechoslovakia to develop means to prolong the effect of the most effective chemical radio-protectors and to find new long-lasting agents. In particular, Bulgarian and USSR scientists created complex compounds of a number of radio-protectors with ion-exchange resins, as well as preparations in which low-molecular chemical radio-protectors are packed in polyvinyl gel. In this latter variant, in order to maintain a stable concentration of radio-protectors in the body for a long time, it was taken into account that this depends on the rate of decay of the polymer which protects the radio-protector from metabolic changes in the mammalian body.

Using this method of prolonging the effect of radio-protectors, it was established that β-mercaptoethylamine protected in a polymer cell is tolerated by the body in higher doses (2-3 times). After the administration of several variants of these complex compounds, β-mercaptoethylamine appears in radio-protective concentration in the blood and organs after 1 hour and circulates in such amount for 5 hours. These data were obtained by Bulgarian scientists in tests on rats with the use of sulfur and carbon labelled cystamine [112]. An important addition to these studies was the research of Soviet scientists investigating the radio-protective effect of the polymer salt of cystamine and sulfopropyl ether of dextran [50].

As the result of these works it was ascertained that the combination of cystamine and sulfopropyl ether of dextran will increase the protection of radiated animals (mice). This appeared especially clearly with the administration of polymer salt of sulfopropyl ether of dextran and cystamine in doses of 500 mg/kg 10-15 minutes before radiation. In these tests, the amount of the cystamine base could be reduced to 62.3 mg/kg which is almost half the radio-protective dose of this substance administered in the form of chloride.

The advantage of a polymer preparation based on dextran and cystamine is that the duration of its effect in comparison with cystamine dichlorhydrate is much longer. Thus, a sufficiently high prophylactic effect was found from a compound of polymer salt of sulfopropyl ether of dextran and cystamine (increased survival 40-70%) administered 1 hour before radiation. An analogous protective effect with the use of cystamine dichlorhydrate can be obtained only when it is administered 4-10 minutes before radiation.

An explanation of this increased duration of the effect of cystamine when combined with sulfopropyl ether of dextran could be that the two amino groups of cystamine form a simple salt compound with the polymer. Evidently, the polymer carrier plays the role of depot, regulating the uptake of cystamine in the blood and then in other organs. Cystamine is gradually separated from sulfonated ether of dextran as the result of ion exchange and appears in the

blood in an amount which is not toxic but already provides effective protection against ionizing radiation for 1 hour.

Studies searching for new long-lasting chemical radioprotectors were conducted by Czechoslovakian scientists. Principal attention was paid to sodium fluoacetate, which in comparison with cystamine, cysteine, AET and serotonin /196
has a longer radio-protective effect and therapeutic range. The protective effect of fluoacetate in tests on mice radiated with x-rays (LD_{80}-LD_{100}) appeared even with administration 1-2 hours before radiation.

According to a test of survival of radiated animals with use of the indicated drug in doses of 5-6 mg/kg, the reduced dose factor (RDF) was 1.44-1.57 [102, 107, 119, 135].

The above mentioned research has made it possible to determine several new chemical radio-protectors which should be subjected to further more careful study primarily during prolonged radiation. This aspect of the work is especially important as recent studies have clearly shown the characteristics of the biological effect of ionizing radiation for a prolonged time and accurate information is lacking on the effect of chemical radio-protectors in case of corpuscular radiation or prolonged radiation at 1 R/min or less, i.e. with radiation injuries which could develop during a space flight primarily because of chromospheric solar flares [3, 29, 96, 97]. In connection with this we undertook additional studies to analyze the effect of the best-known chemical radio-protectors in relation to dose rate, especially the effect of ionizing radiation at a rate of 1 R/min. or less. As the result of these studies it was established that the effectiveness of β-mercaptoethylamine, β-mercaptopropylamine, β-mercaptoethylamine disulfide (cystamine), β-mercaptoethylisothiourea and other compounds at a low rate is considerably reduced or does not appear at all [33, 47].

Taking into account these data on the ineffectiveness of chemical radioprotectors during prolonged radiation, in recent years works have been begun

in the "Interkosmos" program to search for new ways of preventing such injuries. Primary attention was given to the search for radio-protectors and development of anti-radiation formulas which could have a positive effect on the reparation system of the DNA molecule in cells of radio-sensitive organs and tissues and prevent potential changes in the genetic apparatus of cells from becoming irreversible.

An important result of this work was the list of recommendations in relation to the use of ATP and amitetravit preparations which in combined use had the highest radioprotective effect in cases of prolonged, chronic and fractionated radiation.

Along with the search for means to prevent radiation sickness there was no doubt about the need to search for means of pathogenetic treatment of radiation sickness. The most convincing materials were obtained by scientists of socialist countries in validating a method of autotransplantation of bone marrow in case of brief or prolonged radiation (simulating radiation injuries which could arise with powerful chromospheric solar flares). Results of these studies are given in a special section of the present monograph.

In discussing other possible methods of early treatment of radiation injuries, attention was turned to the fact that recent studies have presented proofs of the reality of recovery of the chromosome complex, damage to which was previously considered irreversible [44, 89]. An important prerequisite /197
for studies to search for means of early pathogenetic treatment of acute radiation sickness was information that potential cytogenic damages develop first and a significant portion of them are realized, changing to irreversible form during division of the nucleus in the radiated cell. During the time from the moment of radiation until the start of cell division processes to repair potential damage can occur and there is the possibility of controlled intervention to stimulate recovery processes [63]. The duration of the reparation period — from the end of radiation until the first mitosis — acquires positive value for maintaining cell viability.

Workers have recently established that reparation processes could be significantly changed by intense accumulation of plastic material in the cell, the creation of conditions for active energy metabolism, activation of DNA and protein synthesis and normalization of other biochemical processes [18, 19, 72].

Guided by these reports, scientists of socialist countries, working in the "Interkosmos" program, have concentrated their attention on studying means of treatment primarily able to modify the course of bioenergetic and biosynthetic processes and activate enzyme systems of the recovery of genetic structures. Studies have, therefore, been made on a large number of different chemical compounds and methods of therapy (nucleic acids and several of their analogs, adenosine triphosphoric acid, several of its derivatives and precursors, cocarboxylase, pteridine derivatives, hypoxic hypothermia, etc.).

The most comprehensive studies were conducted by Polish, Czechoslovakian and USSR scientists to determine the advisability of using DNA as a means of early pathogenetic treatment of acute radiation sickness [13, 20, 92, 109, 110]. The greatest contribution to the development of this problem was made by Polish scientists under the guidance of T. Vilchok and Czechoslovakian scientists directed by Z. Karpfel' [70, 83, 92, 93, 120]. Analysis of these works indicates convincingly that a single parenteral administration after radiation during the first 24 hours to small laboratory animals of homologous and heterologous high-polymer DNA with a molecular weight of $10 \times 10^6 - 16 \times 10^6$ can help increase the survival of animals 20-70%. It was also established that parenteral administration of native high-polymer DNA of syngenic origin has a favorable effect on the recovery of bone marrow, increases mitotic activity in bone marrow, helps protect stem hematopoietic elements, accelerates proliferation of cells of damaged bone marrow and reduces the frequency of chromosome aberrations induced by radiation [93, 120].

It was proved that the effect of DNA is most clearly recorded with radiation in doses of LD_{50}-LD_{80}. With large doses (LD_{90}-LD_{100}) the therapeutic effect from its use on small laboratory animals was very weak or completely

disappeared. It was shown that the therapeutic effectiveness of DNA largely depends on its polymer and native characteristics. Several characteristics of the therapeutic effect of DNA also appear with the use of isologous and heterologous DNA. According to data of Czechoslovakian scientists, the most favorable effect is found from the use of isologous DNA. Its therapeutic effect on /198
the hematopoietic system is greater than heterologous DNA. It was established that heterologous DNA does not reduce the level of chromosome aberrations induced by radiation. Comparison of DNA specimens obtained from normal and tumorous tissues, according to data of Czechoslovakian scientists, shows that DNA extracted from tumor tissue has no therapeutic effect.

As a result of physicochemical changes in the macromolecule of exogenic DNA, its therapeutic effectiveness is, as a rule, reduced. It has been shown that thermal denaturation, denaturation by dissolution of DNA in distilled water and other actions completely removed its therapeutic effect in cases of acute radiation sickness [83, 120].

Also established was the absence of any anti-radiation effect from the use of apurine or apyrimidine DNA. Considerably less effective in comparison with high-polymer DNA was post-radiation use of its enzymatic hydrolysates and mononucleotides.

Studies of Czechoslovakian scientists [92-93] revealed that the use after radiation of thymidinic and desoxycytidinic acids gives a reliable therapeutic effect. The least therapeutic effect was with the use of desoxyadenylic acid. The other nucleotides showed no positive effect or even aggravated the process of post-radiation recovery. It was shown that preparations of the nucleotide group have the most pronounced therapeutic effect in doses of 0.2-1 mg per mouse. The effect was reduced when used later than 24 hours after radiation.

It was also found that with the administration of DNA mitotic activity of bone marrow cells is increased, appearing in accelerated normalization of the hematopoietic system. On the basis of histological tests of bone marrow

it was shown that after the administration of pyrimidinic deoxyribonucleotides, proliferative processes are stimulated in stem hematopoietic cells. With the use of such compounds, DNA synthesis is activated in radiated bone marrow, primarily in reticular cells, and a larger amount of endogenic colonies are found in the spleen.

Deserving special attention are data of studies of Czechoslovakian scientists confirming the possibility of obtaining a more pronounced therapeutic effect from the use of exogenic DNA treated ultrasonically than from the use of nucleosides and nucleotides [93]. In addition, it was shown that this treatment of exogenic DNA does not change the biological activity of the macromolecule. In all probability, the cause of the phenomenon is preservation of the double-helix structure of DNA with a simultaneous reduction in the molecular weight to $^{1}/_{20}$ the initial value.

Definite prospects for the treatment of radiation injuries were also revealed from the use of combined DNA and DEAE of dextran which, evidently, helps DNA penetrate the macromolecule in the recipient cell. In all these studies, the favorable effect of exogenic DNA and its precursors was seen on the blood system.

Special works undertaken by Soviet scientists to study the mechanism of this phenomenon showed that the administration of DNA and several of its precursors (thymidine) does not essentially affect the direction of differentiation of stem hematopoietic cells. At the same time they intensify endocolonization of the spleen in unevenly radiated (partially screened) mice [53]. In addition, intensification of erythroblast proliferation was noted under the /199
effect of such drugs. There is basis for assuming that the increased number of endogenic colonies in the spleen of mice radiated while part of the bone marrow was screened with the administration of DNA is due to the increased migration of colony-forming elements from protected bone marrow.

Results of all these studies indicate that in further investigation of the mechanisms of DNA special attention must be given to works interpreting

its effect at the molecular level. In the opinion of Czechoslovakian scientists, the favorable post-radiation effect of exogenic DNA results from the fact that the macromolecule is deposited for a long time in the damaged cell and by means of gradual degradation becomes the substrate necessary for DNA synthesis and recovery of chromosome damages [93, 120]. However, it must be noted that the very fact of DNA penetrating the cell has not been experimentally verified.

In discussing possible mechanisms of the effect of DNA there is basis for assuming that reparation of DNA in cells damaged by radiation with the help of exogenic DNA can result from the use of relatively polymeric proteins to replace damaged sections of DNA-casein or by means of the adaptive increased activity of enzyme systems of reparation.

It is also advisable to subject to further experimental study the possibility of the participation of DNA in replicative synthesis. The possible use of decay products of exogenic DNA as substrates in the biosynthesis of DNA must be kept in mind. It is not ruled out that with the administration of exogenic DNA, competition will arise between it and free DNA of cells for depolymerizing enzymes and thus the effect of DNA-ase activated by radiation will be neutralized.

The mechanism of the therapeutic effect of DNA in cases of acute radiation sickness is still not sufficiently clear. Nevertheless, these works indicate the need for further study of DNA as a means of early treatment and indicate also the necessity of increasing works in this area.

The second direction in studying means of early pathogenetic treatment was investigation of drugs able to activate bioenergetic and biosynthetic processes. Works were conducted primarily to evaluate the effect of ATP, AMP, cyclic AMP and formulas consisting of cocarboxylase and tryptophan, as well as pteridine derivatives.

In joint works by scientists of Bulgaria and the USSR, tests on mice radiated with x-rays in doses of LD_{80} showed the favorable therapeutic effect of cyclic 3'-5'-monophosphate given 10-15 minutes after radiation. Also established was the most positive effect of adenosine-2(3')-monophosphate in tests on radiated rats. The pronounced therapeutic effect of adenosine triphosphoric acid was shown in various kinds of radiation injuries [22, 38, 104].

Considering possible mechanisms of the anti-radiation effect of these compounds, we suggest that they can to a certain degree promote recovery of genetic structures, primarily DNA. This can be partially connected with the ability of monophosphates to prevent disturbances of DNA synthesis, stimulate the formation of enzymes of reparation, prevent disturbances of oxidizing phosphorylation processes, i.e. in other words, to induce reciprocal intra- and inter-chromosome exchanges in the cell which mobilize regenerating mechanisms [63]. /200

In the light of these data, special attention is due facts indicating the ability of AMP and cyclic AMP to prevent changes in various links of metabolism in early stages of radiation sickness. Therapeutic use of AMP in cases of acute radiation sickness established the favorable effect on the deamination of nitrogen bases [9].

Taking into account data on the importance for recovery of genetic structures altered by ionizing radiation, modification of the energy balance of cells and activation of enzyme systems of recovery, it seemed advisable to use as a means of early treatment, formulas consisting of cocarboxylase and tryptophan and pteridine derivatives. These works were conducted by scientists of the USSR, Romania and Czechoslovakia. An important prerequisite for these works was the study by Romanian scientists of the ability of cocarboxylase in combination with tryptophan to activate the synthesis of DNA and RNA and to change the course of conjugated phosphorylation processes leading to accumulation in the cell of energy-rich pyrophosphate bonds [82, 94]. In experiments on mice radiated in doses of LD_{30}-LD_{80} it was shown that this combination,

given 2 hours after ionizing radiation, reduces the number of cells with chromosome damage in bone marrow an average of 20% and increases survival of the animals 40-50%.

Studies of pteridine derivatives [2-amino-4-hydroxyl-6-(1'-2'-, 3'-, 4'-tetrahydroxylbutyl)-pteridine and 2-amino-4-hydroxy-6-(1', 2', 3', 4'-tetrahydroxybutyl)-5-, 6-, 7-, 8-tetrahydropteridine)] showed that parenteral administration of several of these to mice 5-10 minutes after radiation (LD_{100}) has a pronounced therapeutic effect, increasing survival of the animals 20-30% and weakening changes in the blood system. In the opinion of Czechoslovakian scientists, this effect is connected with normalization of oxidation-reduction processes after radiation. The most pronounced effect was recorded from the use of 2-amino-4-hydroxy-6-(1'-, 2'-, 3'-, 4'-tetrahydroxybutyl)-5-, 6-, 7-, 8-tetrahydropteridine [122].

To modify metabolic processes after radiation, taking into account that any delay in cell division during the post-radiation period can aid recovery processes, Czechoslovakian scientists made an attempt to use hypoxic hypothermia as a means of early treatment of radiation sickness [117]. After numerous tests on mice it was established that hypoxic hypothermia created by gradually reducing atmospheric pressure to 200 mm. Hg and lowering the temperature of the environment to 16° has a pronounced effect as a means of early treatment — 30-50% increased survival of the animals (with LD_{70}-LD_{90}), protection of stem hematopoietic cells and a favorable effect on changes in the hematopoietic system. The best results were found in conducting the hypoxic hypothermia session for 6 hours, 2 hours after the end of radiation [100].

In studying other variants of hypoxic hypothermia in tests on mice (reduction of body temperature to 25-30°, pressure to 250-300 mm. Hg), less pronounced therapeutic effects were obtained. Thus, the use of this hypothermia /201 in the first 3-5 days after radiation (650 R) leads only to fewer changes in the blood system.

Numerous studies conducted with other means of early treatment (platinum cis-dichlordiamine, fluoacetate, etc.) of acute radiation sickness give no positive results.

Works searching for means of early pathogenetic treatment of radiation sickness, therefore, in our opinion, indicate good prospects for the development of studies in this area. At the same time they justify the use of early pathogenetic treatment and indicate the correctness of this direction. However, at present there is no doubt that the use of such agents alone will hardly provide sufficiently effective treatment of radiation sickness. The complexity and diversity of pathogenetic mechanisms responsible for the development of radiation sickness indicate the necessity for developing complex pathogenetic therapy which must include besides means of early treatment antibiotics, vitamins, hormonal preparations, stimulants and normalizers of hematopoiesis and anti-hemorrhagic agents.

Works of Soviet scientists showed that the effectiveness of early treatment can be raised with administration in the latent period and at the height of radiation sickness of additional stimulants of hematopoiesis such as leukocytine and leresone [31].

Studies of Czechoslovakian scientists validated the use of ellagic acid in cases of acute radiation sickness as a new anti-hemorrhagic agent [95, 116]. The basic mechanism of this preparation is connected with the activation of coagulation factor XII. In addition, according to the Czechoslovakian data, ellagic acid can be considered a drug with a pronounced anti-heparin property. Ellagic acid reduced symptoms of increased blood flow in tests on radiated rats, guinea pigs and rabbits.

Studies on development and approval of means of treating radiation sickness, therefore, revealed several sufficiently effective anti-radiation drugs on the basis of which development of complex therapy of various kinds of radiation injuries could later begin.

In conclusion we must point out that studies in the area of pharmacochemical protection against ionizing radiation conducted in the "Interkosmos" program made possible several preliminary recommendations in relation to prophylactic and therapeutic measures in case of possible radiation injuries during space flight, in particular, those during chromospheric solar flares. Of greatest interest, in our opinion, might be several prophylactic agents (amitetravit, ATP, prodigiosin, anabolic steroids and other biological protective agents) which can increase not only radioresistance but also the resistance of the body to other extreme factors inherent in space flight. There is basis for assuming that the use of these groups of prophylactic agents in the preflight cosmonaut training period, during the flight when radiation danger threatens and in additional therapeutic administration will create a reliable /202 radiation safety system for space flights. However, more thorough validation of such a system will necessitate additional clinico-experimental studies under ground and flight conditions.

There is no doubt that the productivity of cooperation between socialist countries in the "Interkosmos" program, making it possible to amass a large amount of extremely interesting and important data, on the prevention and treatment of radiation injuries, will help future successful solution of new problems in this area of the creation of a reliable radiation safety system for space flights.

N75 14428

The Biological Effect of Prolonged Radiation and Ways of Selecting New Anti-radiation Drugs Effective in this Kind of Radiation Injury[1]

Recently the number of studies dealing with the biological effect of prolonged radiation, i.e. radiation at a low dose rate — less than 1-5 R/min. — have increased significantly. Interest in prolonged radiation effects is due

[1]The authors are grateful for the highly-skilled help of Z.M. Avdoshin and V.A. Chernikov.

to a number of factors, one of which is the possibility of prolonged radiation in space during solar flares [118, 10]. On a theoretical level, attention is being paid to prolonged radiation because as the rate of radiation is reduced, tolerance of radiation injury by radiated animals increases or, in other words, radiation doses, equally effective in short-term survival test, increase 1½-2 times [85, 130, 103, 71, 11].

Our experiments on random bred mice confirmed the basic characteristics of prolonged radiation [56]. Actually, mortality of mice exposed to γ-radiation at high (600 R/min.) and low (0.28 R/min.) dose rates depended not only on radiation dosage, but also on dose rate (Fig. 46). Comparison of dose-effect lines showed that the values of doses, equally effective according to the lethality index, differed and their ratio in the mid-lethal range was 1.36.

Establishment of mechanisms accompanying the increased radio-resistance of animals during prolonged radiation is an important biological problem. Its solution should be sought first of all in comparing the damage of critical organs and tissues — in hematopoietic and lymphoid organs.

Radiation of animals at both high and low dose rates caused pronounced damage to bone marrow. The number of nucleated cells remaining in the femur and determining the degree of radiation injury in both forms of radiation was exponentially dependent on dosage. In Fig. 46 are represented regression lines of nucleated cells in bone marrow after brief and prolonged radiation. The ratio of regression coefficients characterizing the ratio of equally-effective doses was 2.1.

As the result of prolonged and brief radiation in the doses used, pronounced depopulation appeared not only in bone marrow but also in the thymus, spleen and circulating blood. Post-radiation cytopenia of hematopoietic organs was altered by repopulation processes. The kinetics of these processes, characterized by the rate and quantitative limits of devastation and recovery, /203

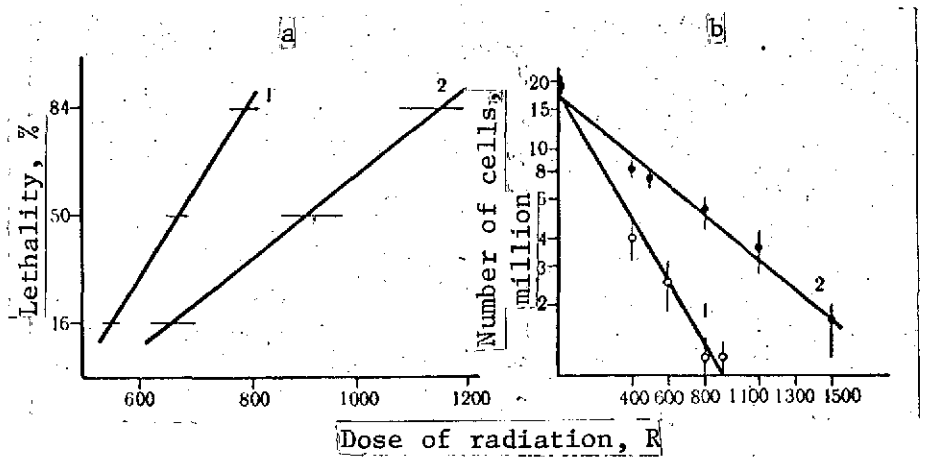


Figure 46. Dependence of mortality of mice for 45 days and cellular content of bone marrow on dosage of brief and prolonged radiation.

a — lethality; 1 — brief radiation; 2 — prolonged radiation; b — cellular content of bone marrow in femur: 1 — 3 days after radiation at dose rate 600 R/min; regression equation $\lg N_1 = 1.2141-0.1386\ x$; 2 — ½ hour after radiation at dose rate 0.28 R/min.; regression equation $\lg N_2 = 1.2469-0.0654\ x$ where N is the number of nucleated cells corresponding to radiation dose x; at the dot 10-25 mice were studied. Horizontal and vertical lines — confidence intervals.

could be correctly compared only by taking into account individual hematoblasts, as changes in each one have their own temporal and quantitative parameters. Comparison of quantitative hematological indices of mice radiated briefly and for a long time in equivalent doses of 800 R, causing the death of 80 and 39% of the animals respectively, was made, as well as those of equally-effective lethal doses — 800 and 1100 R (LD_{80}). Hematological changes developing after brief and prolonged radiation in doses of 400 R, causing no lethal outcomes, were previously analyzed in work [59].

The erythroid system. After radiation in doses of 800 R significant changes developed in red bone marrow. The reduction in erythroblasts was swift to an indeterminate level. Recovery of the number of erythroblasts in

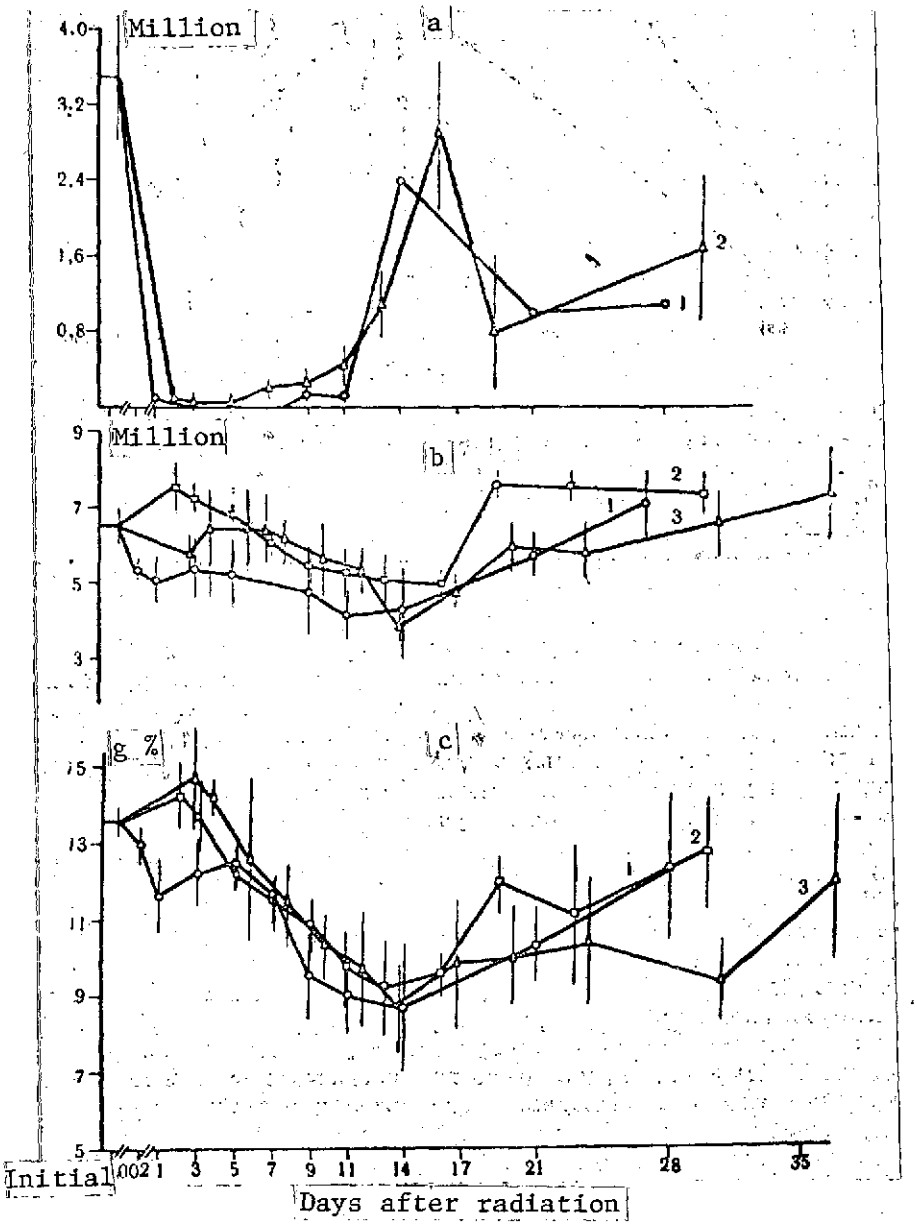


Figure 47. Changes in the number of erythroblasts in bone marrow, erythrocytes and hemoglobin content in the blood of mice radiated briefly (1) and for a long time in doses of 800 R (2) and 1100 R (3). /204

a — number of erythroblasts in bone marrow of femur; b — number of erythrocytes in 1 mm^3 of blood; c — hemoglobin content. Vertical lines — confidence intervals.

bone marrow after prolonged and brief radiation began on the 7th and 9th day respectively (Fig. 47). In some of the animals after prolonged radiation reticulocytes in the blood were determined continuously. In case of brief radiation reticulocytes disappeared from the blood stream completely and appeared again in small numbers after 9 days. Intensive recovery of the number of reticulocytes in all test mice was noted after the 11-13th day. As a result of the intensive recovery process, by the 19th day the initial level (176±26 thous.) was exceeded 3 times in mice radiated for a long time and twice in those radiated briefly. With regard to erythrocytes, their number in mice radiated for a long time was 1.5-2 million higher than in those radiated briefly at all points of the study. There is interest in the fact that brief erythremia was observed after termination of prolonged radiation and then for 8 days — erythropenia. In case of brief radiation, initial erythremia was not noted and the period of erythropenia lasted up to 3 weeks. /205

The concentration of hemoglobin changed basically in accordance with the number of erythrocytes (see Fig. 47). Reduction in the concentration of hemoglobin in mice radiated briefly was more significant. The difference from the group radiated for a long time in minimum concentrations, registered in 13-14 days, was 1 g%. Recovery of the concentration of hemoglobin was traced through the 3rd and 4th weeks; the concentration of hemoglobin in mice of both groups lagged behind the number of erythrocytes appearing in the blood, i.e., hemoglobin saturation of reformed erythrocytes was less than initially. As an example, on the 21st day color indices in mice radiated for a long time and briefly were 0.46 and 0.56 respectively and in intact animals — 0.63. Therefore, changes in the erythron system, caused by brief and prolonged radiation in equivalent doses of 800 R, differed distinctly. With these kinds of radiation in doses of 800 and 1100 R respectively no important differences were revealed in red blood indices (see Fig. 51).

Myeloid system. After the effect of comparable radiation in doses of 800 R, the decrease of cells in the proliferating pool, including myeloblasts, promyelocytes and myelocytes, was similar (see Fig. 53). On the 3rd day and

later throughout the recovery period the number of cells in the proliferating pool was greater in mice radiated for a long time than in animals radiated briefly.

The number of metamyelocytes and rod neutrophils in the femur of mice radiated for a long time, throughout the entire period of post-radiation cytopenia, exceeded the number of elements in the maturing pool of briefly radiated animals by 0.2-0.3 million.

Mature neutrophils decreased in the bone marrow of animals in the compared groups differently (Fig. 48). In mice radiated for a long time, the minimum level was recorded 2 days later — at the end of the 5th day after radiation. During the next 10 days the number of neutrophils in mice exposed to prolonged radiation fluctuated only very slightly. In this same period in mice radiated at a high dose rate there occurred a brief increase in the number of neutrophils and secondary devastation by the 11th day.

The recovery of the number of neutrophils in bone marrow in case of prolonged radiation proceeded swiftly. The number of segments increased 22 times in 3 days, significantly exceeding not only the number of this kind of cell in mice radiated briefly, but doubling the initial level.

The number of mature neutrophils in bone marrow changed in accordance with their kinetics in bone marrow (Fig. 48). Reduction in the number of neutrophils in blood after prolonged radiation was delayed 2 days; depopulation was not preceeded by the radiation neutrophilia reaction which occurred in mice exposed to the effect of radiation at a high dose rate. The level of developing agranulocytosis did not depend on the kind of radiation used. Along /206
with this the exceptional intensity of myelopoiesis in animals radiated for a long time was favorably reflected in the recovery of granulocytes in the blood. On the 16th day the number of polymorphonuclear neutrophils in peripheral blood of mice radiated for a long time had already reached the initial level and by the 19th day exceeded it more than twice.

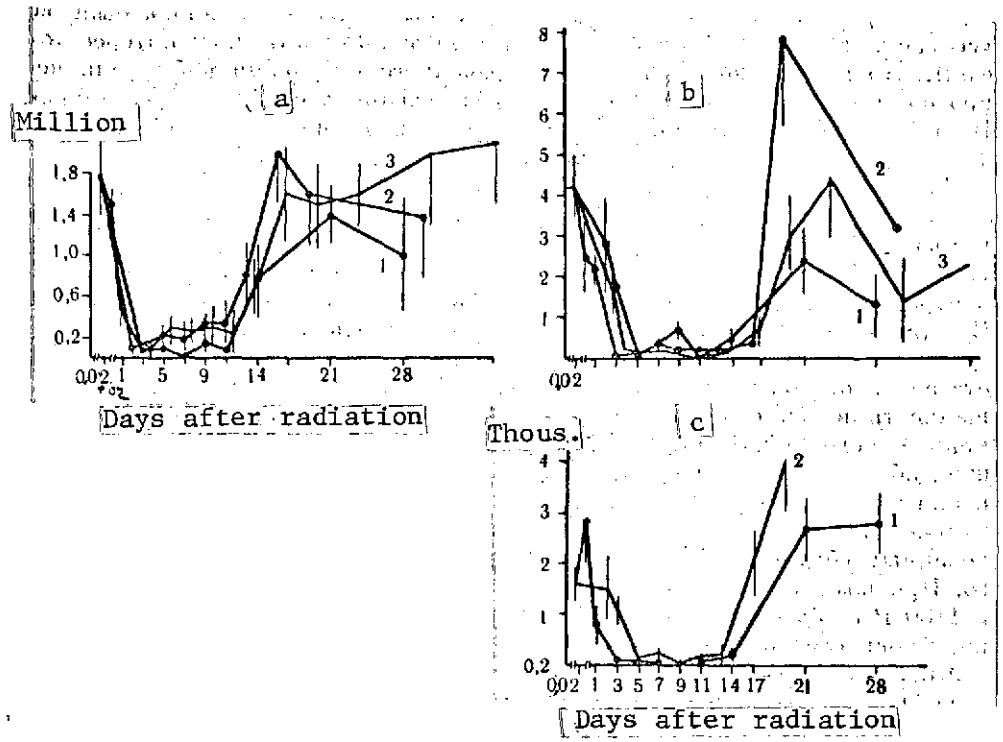


Figure 48. Changes in the number of myeloid cells in mice radiated briefly (1) and for a long time in doses of 800 (2) and 1100 R (3)

a — number of myeloblasts, promyelocytes and myelocytes in bone marrow of the femur; b — number of polymorphonuclear neutrophils in bone marrow of the femur; c — number of polymorphonuclear neutrophils in 1 mm^3 of blood. Vertical lines — confidence intervals.

In conclusion it can be noted that with radiation at a low dose rate, the times of depopulation in bone marrow and blood are lengthened, the reaction of post-radiation neutrophilia is not recorded, the severity of cytopenia is reduced because of elements of all degrees of maturity and the rate of repopulation of granulocytes rises; as a result, in the time allotted for the study, a more complete recovery of myeloid growth is reached in bone marrow and mature elements in peripheral blood.

With prolonged and brief radiation, equally effective according to the index of survival (LD_{80}), similar pictures of depopulation and recovery of myeloid growth were registered in test mice (see Fig. 48).

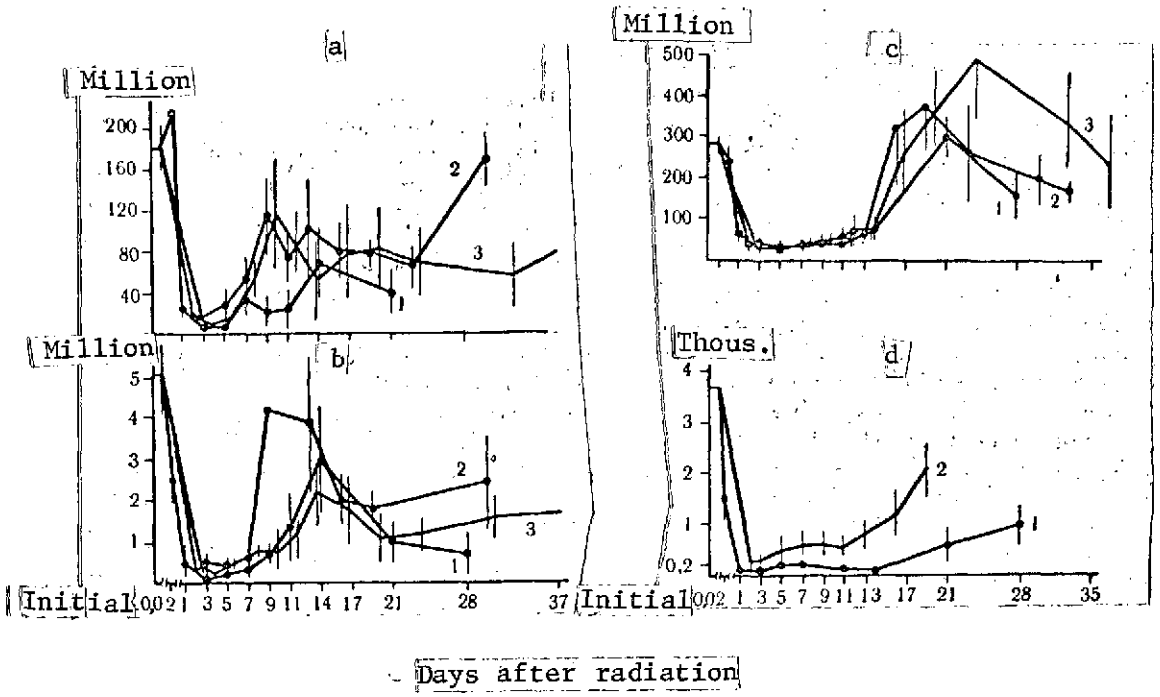


Figure 49. Changes in the number of lymphoid elements in mice radiated briefly (1) and for a long time in doses of 800 R (2) and 1100 R(3).

a — number of thymocytes; b — number of lymphycyte-like cells in bone marrow of the femur; c — number of nucleated cells in the spleen; d — number of lymphocytes in 1 mm^3 of blood.

Lymphoid system. The kinetics of lymphoid elements were studied at the same time in the thymus gland, bone marrow, spleen and peripheral blood. Analysis of changes in these tissues gives a definite picture of the damage to the system, the rate and fullness of recovery, as well as the conjugation of these processes in the organs.

Depopulation of the thymus after prolonged and brief radiation in doses of 800 R lasted for 3 days (Fig. 49). By the time the process of depopulation was completed in mice radiated for a long time, the number of thymocytes was double that of animals radiated briefly. Differences in cell content in favor of prolonged radiation were also traced at all succeeding points of the study. In

the recovery period, especially on the 9th day, they reached maximum. The cell count of the thymus in mice radiated for a long time exceeded that index in animals radiated briefly 5 times. The cell count registered during repopulation was half that of the initial level. At this level the number of cells seemed to stabilize until the 21-23rd day. Only by the 30th day did the cell count of the thymus in mice radiated for a long time reach the initial level. In animals radiated briefly the initial level was not reached during the time set for the study.

The number of lymphocytes in bone marrow (5.1 million) was reduced after prolonged and brief radiation in doses of 800 R 16 and 51 times respectively (see Fig. 49). Recovery of the number of lymphocytes began earlier, on the 2-3rd day after the start of radiation and lasted for 10-11 days, showing an exponential dependence on time $\lg L_{pr} = 1.1486 + 0.1338\ t$ and $\lg L_{br} = 0.5653 + 0.1398 \cdot t$, where L is the number of lymphocytes in bone marrow t days after /208 the start of prolonged (pr) or brief (br) radiation. For the recovery period the number of lymphocytes increased to 3-4 million. After 16 days, a second reduction was noted in the number of lymphocytes contained in bone marrow of mice radiated for a long time or briefly was stabilized at a level half that of the initial.

Temporal and quantitative parameters of depopulation of the spleen after prolonged and brief radiation in doses of 800 R coincided (see Fig. 49). The number of splenocytes in the first 2-3 days after the start of radiation was reduced 7-9 times and was stabilized at this level for 10 days. The number of cellular elements was restored after 11 days and in the period from the 13th to the 16th day increased 5 times, exceeding initial levels. However, repopulation of the spleen during intensive recovery was due not to lymphoid elements whose number was determined at a level half that of the initial. The 80% recovery of cell count during this period was due to myeloid and erythroid elements.

The number of lymphocytes in circulating blood was reduced 14 times during prolonged radiation in doses of 800 R. During the entire succeeding observa-

tion period it increased steadily and by the 19th day reached only half the initial level (see Fig. 49).

As a result of brief radiation in the same dosage the number of lymphocytes was reduced by the end of the first day 37 times. The minimum level of lymphocytes (0.1 thousand per 1 mm^3) was recorded during the next two weeks. An increase in the number of lymphocytes was established on the 21st and 28th days; however, it was insignificant — 6 and 3.7 times less than the initial level respectively. In other words, the number of lymphocytes circulating in the blood of animals radiated for a long time was greater than in mice radiated briefly at all times of the study. This difference increased with the passage of time after radiation. Thus, 1-3 days after prolonged radiation the number of lymphocytes was 2-3 times greater than with brief radiation and on the 9-14th day, 5-6 times higher. Therefore, the content of thymocytes and lymphocytes in bone marrow and in the blood of mice exposed to prolonged radiation was at all stages of the study higher than in animals radiated briefly; this constitutes one of the differences in the biological effect of compared radiations in doses of 800 R at different dose rates. It is also necessary to note that comparative evaluation of brief and prolonged radiation in doses of 800 and 1100 R showed no differences in the kinetics of lymphoid elements (see Fig. 49).

The system of stem elements. The method of quantitative determination of colony-forming elements (KOE) presented new possibilities for studying the stem pool of hematopoietic tissue. The authors of the McCulloch and Till method traced in detail the kinetics of the damage [101] and recovery [131] of KOE in mice exposed to brief radiation. Changes in KOE in animals radiated for a long time are described in works [59, 55]. The results of studies conducted on F_1 hybrid mice demonstrated the difference in the damaging effect of brief and prolonged radiation, as well as in the rate of post-radiation repopulation of KOE. These differences are important in interpreting the characteristics /209 of prolonged radiation.

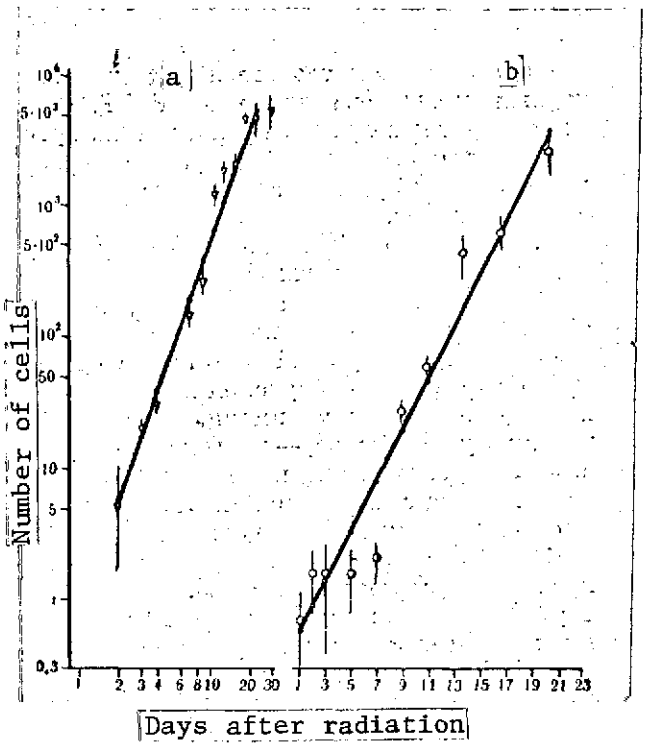


Figure 50. Kinetics of KOE of bone marrow of mice radiated briefly (b) and for a long time (a) in doses of 800 R.

The different signs with vertical lines indicate experimental values with confidence intervals, straight lines — calculated regression lines: a — lg KOE = 0.0882 + 2.8023 lgt; b — lf KOE = 0.4238 + + 0.1895 t.

During prolonged radiation in doses of 800 R, the number of KOE contained in bone marrow of the femur decreased from 4949 (4150-5148) to 5.0 and a day after termination of radiation increased to 20 units. Increase in the number of KOE was traced until the 19th day (Fig. 50). As calculations showed, dependence of the number of KOE on time elapsing after the start of prolonged radiation can be modelled both as an exponential and as a power function. As the result of verification by statistical criterion, it was established that a straight line, plotted on the basis of power dependence, better satisfies the dispersion of experimental points.

Thirty minutes after termination of radiation at a high dose rate 3.7 KOE were determined in the bone marrow of the femur. Therefore, brief and prolonged radiation in equivalent doses first cause approximately the same reduction in KOE. However, 24 hours after brief radiation the amount of KOE not

only did not increase, but also continued to decrease so that by the end of the first day 0.7 units were determined in the femur. Increase in the number of KOE with brief radiation was registered only after 7 days. The process of repopulation of KOE after brief radiation, as in case of a prolonged effect, can be modelled by both exponential and power functions, although verification by statistical criterion shows that in this case a straight line plotted on the basis of exponential dependence better satisfies the dispersion of experimental points. The rate of increase in the number of KOE after brief radiation was higher (see Fig. 50).

The greater number of stem cells, determined in mice radiated for a long /210
time in doses of 800 R, could give them certain advantages in the repopulation of hematopoietic and lymphoid tissue, which were expressed in the earlier and more complete recovery of cellular populations in comparison with mice radiated briefly in equivalent dose.

It is known that KOE in the pool of stem hematopoietic elements are the leading factor in the recovery of hematopoiesis in the radiated organism and, therefore, also in the outcome of radiation injury with a preponderance of bone marrow syndrome. In connection with this, the difference in post-radiation kinetics of KOE in case of brief and prolonged radiation is, evidently, the determining factor. It can help understand the chief characteristics inherent in prolonged radiation — increased resistance of animals to radiation. Resistance can be increased because after radiation at a low dose rate there is no total blockade of the repopulation process, in particular of stem cells in bone marrow.

Other characteristics inherent in prolonged radiation can also be interpreted from this aspect. Radio-protectors, approved for brief radiation conditions, are ineffective in cases of radiation of animals at low dose rates [24, 68, 15, 33, 47].

TABLE 45

RADIO-PROTECTIVE EFFECTIVENESS OF SEVERAL DRUGS IN ONE-TIME INTRA-ABDOMINAL ADMINISTRATION UNDER CONDITIONS OF PROLONGED RADIATION

Drug	Dose mg/kg	Time of administration, hours	Radiation dose, R	Dose rate, R/min.	Number of mice	Survival of mice (%)		
						test	control	P
Polysaccharide-A	50–500	0.5 24	900	0.28	126	30-37	32	> 0.1
Dextran-histidine	3000	24	800	0.28	124	44	52	> 0.1
Prodigiosan	5	24	800	0.28	173	44	49	> 0.1
	5	24	900	0.28	130	23	31	> 0.1
Cystamine	150	0.5	900	3.75	100	40	33	> 0.1
Colchicine	2	0.5-2	1,000	0.28	124	28	24	> 0.1

Analogous results were obtained in our studies (Table 45).

It is evident that the use of protectors (aminothiols, polysaccharides) having a pronounced cytostatic effect in cases of prolonged radiation promotes the disturbance of the KOE repopulation rate. Slowing down the rate can have an unfavorable effect on recovery processes in the blood system and, therefore, also on the outcome of radiation sickness. Verification of this assumption can be presented by studies conducted with colchicine. The latter, as is known, is an inhibitor of mitotic activity. The administration of colchicine for 30 minutes 2 hours before the start of prolonged radiation did not increase the survival of mice, which is usually recorded with brief effects [28]. However, the course of radiation injury, caused by prolonged radiation, in all probability could be affected by accelerating the rate of KOE recovery. On this level the use of drugs capable of stimulating metabolism and proliferation processes can be quite promising. The successful use of ATP to prevent and treat radiation syndrome verifies the possibility of modifying prolonged radiation injury [41]. /211

In our studies, conducted together with specialists of the People's Republic of Bulgaria T. Pantev, N. Bokova and I. Nikolov [26], it was established

TABLE 46

ANTI-RADIATION EFFECTIVENESS OF ADENOSINE TRIPHOSPHATE SALT OF β-MEA IN DOSES OF 750 MG/KG IN PROLONGED RADIATION

Dose of radiation, P	Dose rate, R/min.	Time of administration of drug before radiation, min.	Number of mice	Survival of mice, %	P
835	10.7	20	40	75	< 0.001
		Control	34	29.5	--
900	3.75	20	28	71.5	< 0.01
		Control	30	33.3	

that ATP salts also show small protector properties during prolonged radiation. As seen from Table 46, survival of mice protected by ATP-sale of β-MEA was higher than in the control group by 38-45%.

In conclusion we must note that the basic characteristic of prolonged radiation — increased tolerance of radiation injury — can be explained taking into account cellular kinetics; as dose rate is reduced, the repopulation rate is not disturbed, particularly that of stem cells, which, evidently, makes it possible for the organism to tolerate higher radiation loads. On this basis we must assume that approved radio-protectors whose effect contains an established cytostatic component are unsuitable for prolonged radiation. Evidently, under conditions of prolonged radiation it is better to correct the stem pool formation process by other methods, either by accelerating the proliferation of cells or limiting the effect of stimuli causing cells to lose colony-forming properties.

N75 14429

Experimental-clinical Validation of the Use of Amitetravit, ATP and Autologous Bone Marrow in Radiation Injuries Caused by Prolonged Radiation

Data on the presence of radiation danger in carrying out space flights have necessitated developing anti-radiation drugs and methods of treating radiation sickness in accordance with present ideas of processes responsible for the formation of the natural resistance of the body and of basic mechanisms of damage and recovery in radiosensitive organs and tissues.

The greatest radiation danger during space flight is that of solar flares, i.e., prolonged radiation at a low dose rate. In connection with this, a special significance is acquired by the problem of devising anti-radiation agents which remain effective with this kind of radiation. /212

Our experimental studies were conducted in three directions: 1) the effectiveness of agents raising the natural resistance of the body were studied; 2) the use of adenosine triphosphoric acid (ATP) was verified as an anti-radiation agent in various kinds of radiation injuries; 3) the therapeutic effectiveness of autotransplantation of bone marrow was studied.

Principal attention in studies in the first direction was concentrated on the biological protective agent amitetravit, created on the basis of our concept of the presence in the body of a common protective-restorative system protecting vital structures from damage, regardless of the causative factor.

Amitetravit is a complex of four vitamins: B_1, B_6, C and P and two amino acids: histidine and tryptophan. Studies of its radio-protective, protective-therapeutic and therapeutic effect were made during various kinds of ionizing radiation (γ-rays, x-rays, high-energy protons) on animals (mice, rats, dogs) in cases of acute and subacute forms of radiation sickness in a range of doses from non-lethal to LD_{40-70}.

In all versions of the experiments it was established that the administration of amitetravit moderated radiation injury, reduced shifts in hematopoiesis, encouraged more rapid reparation of the blood system and weakened disturbances in immunobiological reactivity which is an integral index of the general reactivity of the body; survival of the animals increased with lethal doses of radiation [32, 21, 34].

Unlike chemical protective agents which are ineffective with radiation at a low dose rate, the use of amitetravit also had a positive effect with prolonged radiation. Prophylactic and protective-therapeutic administration of the drug to rats and mice in case of 3-4 day radiation at a rate below 1 R/min. increased survival 55-25% with LD_{70-100} [16, 34].

The systematic administration of amitetravit in courses of "chronic" daily γ-radiation of dogs for 3 years (total 62 rad per year) with three additional radiations during the year in doses of 42 rad, simulating the effect of solar flares, preserved the normal quantitative and qualitative composition of bone marrow and peripheral blood during the entire observation period, had a stabilizing effect on autoflora of the body and phagocytic activity of the blood, checked the development of autoimmune reactions, lessened the severity of biochemical and endocrinological changes and had a favorable effect on the function of the vestibular analysor [40].

A clinico-physiological study of the drug, conducted on 70 volunteers, established good tolerance of the drug and lack of side effects. In addition, noted after taking amitetravit was a reduction in vegetative-vascular lability, an increase in the rate of dark adaptation and in a number of persons — an increase of visual acuity in twilight conditions and better tolerance of Coriolis acceleration [14].

These materials convincingly indicate the feasibility of using amitetravit in various kinds of radiation injuries — from brief, fractionated, prolonged and chronic radiation. In addition, the low toxicity of the drug, the /213

favorable effect on the vestibular analysor and immunological reactivity and the general condition of the body indicate its use during radiation in space flights.

Studies in the second direction were based on assumption of the important role of biochemical systems regulating the synthesis of nucleic acids and of oxidizing phosphorylation in the mechanism of damage and recovery of genetic structures.

The idea of the essential significance in radiation damage of the inhibition of oxidizing processes, connected with one of the most important manifestations of life — the production of energy — was first expressed by I. I. Ivanov et al. [17]. Disturbances in conjugated oxidizing phosphorylation cause a deficit of macroergic compounds in tissues, primarily ATP, which could be one cause of disturbance in the most important energy-consuming syntheses in cells, including DNA synthesis [8, 48].

These disturbances in the bioenergy system were ascertained for acute radiation damage caused by brief radiation. However, it can be assumed that they are also the rule for radiation at low dose rates. Experimental data has now been accumulated on the effectiveness of ATP used with brief radiation [138, 36, 38]. It has been shown that ATP has a pronounced anti-radiation property not only with radiation damage in small laboratory animals, but also in larger mammals — dogs [38]. The protective effect of ATP appears in the prevention of changes, primarily in radiosensitive organs and tissues. The prophylactic and protective-therapeutic use of ATP decreased necrobiotic processes and the number of cells with chromosome aberrations in bone marrow [38].

With the use of ATP a correlation is detected between the severity of changes in the hematopoietic system, in the clinical state, in the hemorrhagic syndrome and survival of radiated animals.

Brief radiation of rats with x-rays in doses of 500 R revealed the favorable effect of ATP on gastric secretion [84].

The prophylactic (1 hour before radiation) administration of ATP to rats prevents or reduces post-radiation disturbances in the mitochondria structure and changes in the content of nucleic acids, glycogen and lipids in hepatic cells [139]. The positive effect of ATP in prophylactic or therapeutic administration was also noted with fractionated daily radiation of rats with x-rays in total doses of 1000 and 1500 R [105].

Study of the effect of ATP in cases of prolonged radiation was begun in 1968-1969 in experiments on mice and guinea pigs. It was revealed that with γ-radiation at a dose rate below 1 R/min. and with radiation lasting from 16 hours to several days, several variants of ATP administration encourage a small (19-30%) but reliable increase in survival of radiated animals, although no permanent positive effect could be noted in these experiments [41].

It seemed especially important to find the possibility of using the administration of ATP in cases of prolonged radiation in experiments on dogs, as only tests on large mammals will give reliable basis for assuming that the effect of these agents will be preserved in their use on man. /214

Experiments were conducted on dogs radiated with γ-rays (Cs^{137}) in doses of 700 and 800 R at a rate of 1 R/min. The latter is close or equal to the critical threshold dose rate where chemical protectors have no radio-protective effect. In length of exposure (11 hours 40 minutes — 13 hours 20 minutes) the selected models of radiation can be considered a simulation of powerful solar flares.

ATP was administered intramuscularly to dogs of the test group 10-20 minutes before radiation and 10-20 minutes after on the basis of 35 mg/kg in the form of a 1% solution of sodium salt with the addition of 3-5 ml 2% solution of novocain. The same amount of novocain was given to control dogs. After radiation the animals were subjected to no additional therapeutic procedures.

Observations showed that this variant of ATP administration produces a persistant and quite pronounced anti-radiation effect. With a radiation dose of 800 R, constituting $LD_{96/45}$, survival of dogs in the test group reached 45%, at 700 R ($LD_{70/45}$) — 60% (Table 47).

Table 47

THE EFFECT OF THE ISOLATED USE OF ATP AND ITS COMBINATION WITH THE PROPHYLACTIC ADMINISTRATION OF AMITETRAVIT ON SURVIVAL OF DOGS IN CASE OF PROLONGED RADIATION (1/R/MIN.)

Variant of test	Radiation dose, R	Number of dogs	Survived 45 days	Survival, %	Average life span, days	P (according to survival)
Control radiation	800	25	1	4.0	14.4	
ATP, 35 mg/kg	800	20	9	45.0	17.4	< 0.001
Amitetravit + ATP	800	23	13	57.0	16.8	< 0.001
Control radiation	700	18	5	27.8	14.0	
ATP, 35 mg/kg	700	10	6	60.0	21.1	< 0.001
Amitetravit + ATP	700	14	13	93.0	19.5	< 0.001

The administration of ATP did not completely prevent the development of clinical symptoms of radiation sickness in dogs; however, their frequency and severity were markedly less. It must be noted that in the period of the greatest depression of hematopoietic function the reduction of the leukocyte content in peripheral blood in test dogs was reliably less in comparison with the control, especially with radiation doses of 700 R.

It was interesting and important to study the effectiveness of ATP in cases of prolonged radiation against a background of increased resistance of the body which was provided by the prophylactic administration of amitetravit to the animals. Experiments on dogs were conducted with the same model of prolonged radiation and the same means of administration of ATP used to study the effect of its isolated administration. Amitetravit was given to the dogs for 12-14 days before radiation, twice a day, internally. Results of studies showed that combining the prophylactic administration of amitetravit with the

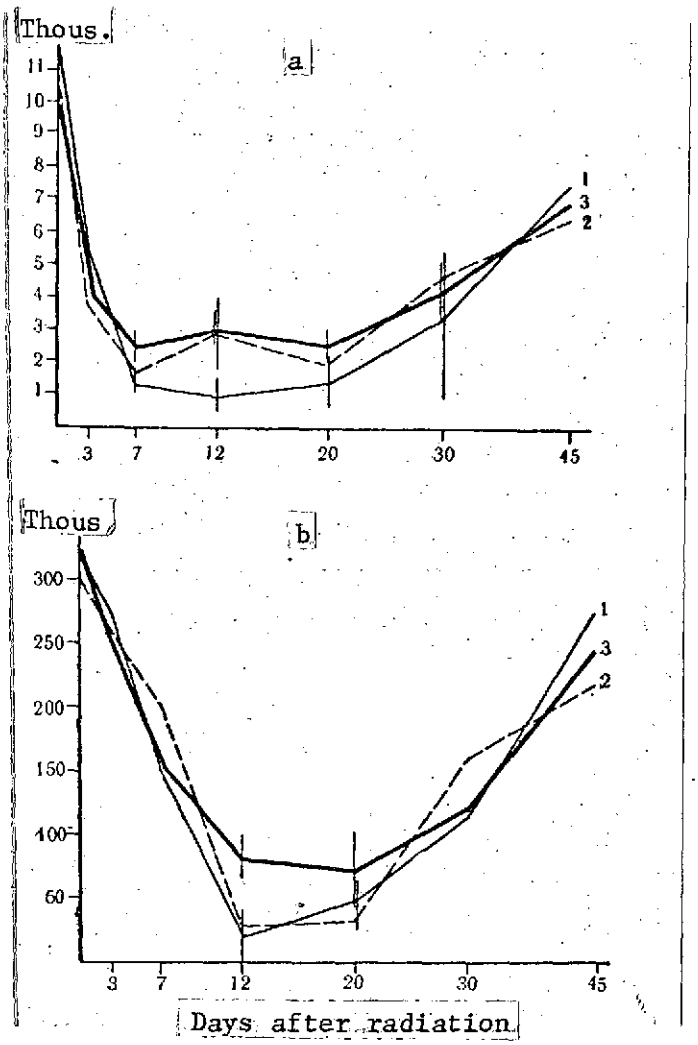
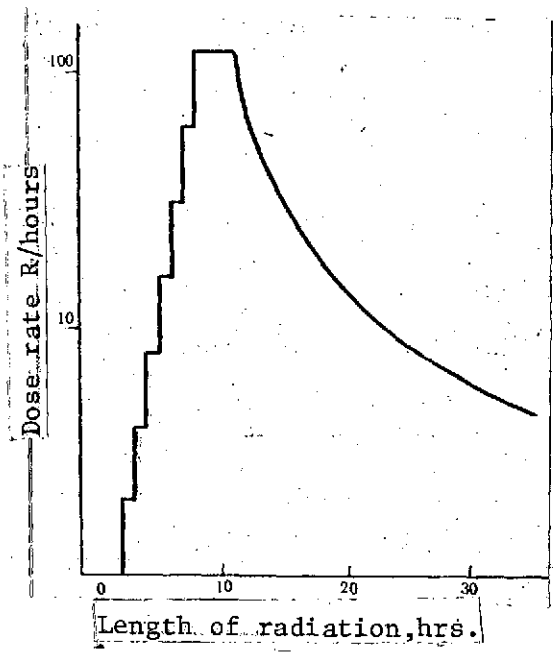


Figure 51. Changes in the number of leukocytes (a) and thrombocytes (b) in peripheral blood of dogs with prolonged radiation in doses of 700 R (R/min).

1 — control radiation; 2 — ATP; 3 — amitetravit + ATP.

protective-therapeutic use of ATP markedly intensifies the effectiveness of of the latter. As seen from Table 47, with radiation in doses of 800 R, survival of dogs increased to 57% with the combined use of ATP and amitetravit. With a dose of 700 R the combined administration of the two drugs was much more effective: 13 out of 14 dogs survived, which is 93%, and exceeded the survival of control dogs 73%. The combination of drugs significantly weakens the severity of radiation sickness and lessens disturbances of hematopoiesis in test dogs in comparison with control animals, especially in doses of 700 R.

With a combination of the two drugs a reliable difference was noted in the level of leukocytes in the period between the 7th and 20th days as well as thrombocytes (Fig. 51). The administration of ATP and amitetravit prevented the elevated level of 11-oxycorticosteroids in blood plasma at the height of the disease which is typical of control dogs. On the 7th and 12th day the content of 11-OCS was 18.5 and 14.9 mg% in the control, in test groups it stayed between 10.2-12.3 mg% (normal 9-7.7 mg%).



/216

Figure 52. Changes in exposure dose rate of radiation in time (R_{init} = = 1 R/hr; R_{max} = = 127 R/hr).

The combined administration of amitetravit and ATP, therefore, significantly increased the survival of animals and improved the clinical course of radiation damage.

The third direction of works is connected with search for means of early pathogenetic treatment and is based on data that successful regeneration of the hematopoietic system to a significant degree depends on the number of preserved stem hematopoietic elements. Most studied at the present time is the effect of myelotherapy [66, 129, 7 and others]. However, because of antigenic differences between the donor and recipient there is the possibility of secondary disease [27, 127]. This can be avoided by the use of autologous bone marrow, highly-effective in tests on dogs and monkeys radiated with x- and γ-rays at high dose rates [54].

Our experiments in evaluating the therapeutic effect of automyelotherapy in cases of prolonged γ-radiation (Cs^{137}) were performed on 31 dogs. On the basis of calculated data and the technical resources of the GUB-1 equipment [6] we worked out a model of 33.5-hour radiation of dogs at a variable rate (from 1 to 127 R/hr) with total dosage of 1000 R, simulating a powerful solar flare. Changes in the exposure dose rate of radiation at the place where the cages with the experimental animals were located, corresponding to the devised model of radiation, are given in Fig. 52.

Four dogs were given transplants of freshly-prepared bone marrow, 13 of preserved marrow kept at the temperature of liquid nitrogen for 4-14 months; 14 dogs served as the control.

Bone marrow for autotransplantation was obtained by means of puncturing both humerus bones. The volume of the suctioned suspension varied between 150-200 ml, the number of myelokaryocytes — $3.1\text{-}8.6\cdot10^9$. Replantation of bone marrow was carried out intravenously by the fast-drip method 1½-2 hours after termination of radiation. Preserved bone marrow was replanted in 7 dogs in amounts /217 of $3.1\text{-}5.9\times10^9$ cells; the other 6 dogs received lower doses of myelokariocytes, $0.85\text{-}1.2\times10^9$. All dogs, those with transplantation of bone marrow and the controls, received in the period 5-20 days after radiation intramuscular injections of antibiotics: penicillin — 300,000 units each and streptomycin — 250,000 units each, twice a day. Basic criteria in evaluating the effectiveness of automyelotherapy were survival, the clinical course of radiation sickness, depth of damage, rate and fullness of recovery of hematopoiesis.

Radiation at a variable rate in doses of 1000 R led to the development of a severe form of acute radiation sickness in all control dogs with pronounced cytopenic and hemorrhagic syndromes and to death between the 9th and 16th days after radiation. Transplantation of bone marrow, both freshly-prepared and preserved, prevented the death of 12 out of 17 treated dogs and markedly alleviated the course of radiation sickness (Table 48; Fig. 53 and 54).

TAble 48

EFFECTIVENESS OF AUTOMYELOTRANSPLANTATION IN DOGS UNDER CONDITIONS OF PROLONGED RADIATION AT A VARIABLE DOSE RATE (1000 R)

Variant of test	Number of transfused bone marrow cells x 10^9	Number of transfused cells per kg weight x 10^9	Percent of viable cells	Time of preservation of cells	Number of dogs in group	Number of surviving dogs
Fresh bone marrow	7.5-8.6	0.37-0.43	-	35-36 hrs	4	4
Preserved marrow	3.1-5.9	0.14-0.26	87-99	4-14 mos	7	6
	0.85-1.5	0.04-0.07	79-99	4.5-6 mos.	6	2
Control radiation	-	-	-		14	0

The dependence of the therapeutic effect on the number of transfused cells was revealed. With replantation of 3.1-5.9 billion myelokariocytes, 6 dogs out of 7 survived and when the number of transfused cells was reduced to 0.85-1.5 billion only 2 out of 6 dogs survived (see Table 48).

Analysis of data indicates that autotransplantation had no essential effect on the depth of cytopenic changes in the blood and hematopoietic organs during the first week after radiation, but aided in the rapid recovery of hematopoiesis (see Fig. 56 and 57).

On the 12th day after radiation, especially with the use of fresh bone marrow, signs of activation of hematopoiesis were also noted: an increase in the total number of leukocytes and thrombocytes and the appearance of reticulocytes in peripheral blood. The number of myelokariocytes increased in punctates of bone marrow, cells of early generations appeared among elements of the red series. By the 20th day, young forms and mitotically dividing cells of the myeloid series appeared. Recovery of the total number of leukocytes in peripheral blood was basically the result of increase in the absolute content of neutrophils. Lymphopenia was preserved much longer. Thus, on the 60th day after radiation, with a normal amount of leukocytes in the blood, the lymphocyte content was only 40-60% of initial values. The total number of

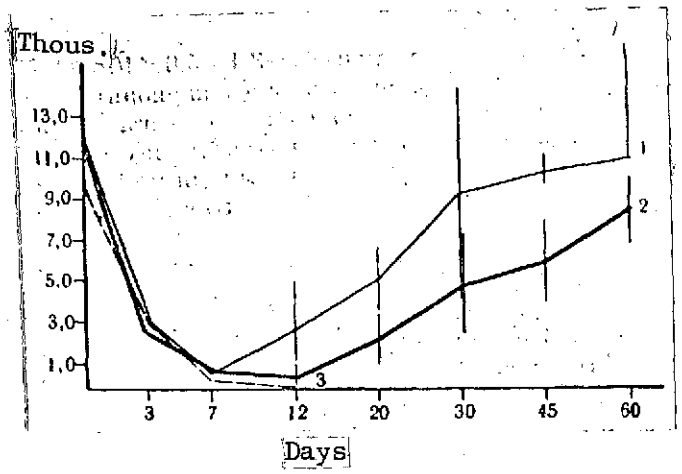


Fig. 53. Changes in the total number of leukocytes in dogs treated with autologous bone marrow.

1 — fresh; 2 — preserved; 3 — control radiation.

erythrocytes and the hemoglobin content changed moderately in the period between the 20th and 30th days. By the 45th day, red blood indices reached normal levels in dogs treated with freshly prepared bone marrow.

The data we obtained on the use of autologous bone marrow in cases of prolonged radiation showed its high therapeutic effectiveness and completely agreed with the experiments of I.L. Chertkov et al., with brief radiation [54]. The cause of the more rapid recovery of hematopoiesis in animals treated with fresh autologous bone marrow in comparison with the preserved material was, in our opinion, besides the use of double the amount of transfused myelokaryocytes, also that mother cells which have withstood the effect of ultra-low temperatures show an ability for proliferation and differentiation 7-9 days later than mother cells of fresh bone marrow [52].

These experimental-clinical studies show , therefore, the validity for space medicine of using amitetravit as a means of increasing natural radioresistance, ATP as a protective-therapeutic drug in cases of prolonged radiation simulating the effect of chromospheric solar flares and automyelotransplantation as a means of early pathogenetic treatment. These materials are deserving of attention as they indicate the possibility of producing a prophylactic and therapeutic effect not only in brief radiation, but also with prolonged ionizing radiation when none of the known chemical radio-protectors

has a reliable positive effect. For space medicine it is also, evidently, important that amitetravit is well-tolerated by animals and man; it causes no side toxic reactions and raises resistance of the body not only to the effect of ionizing radiation, but also to several other extreme stimuli (acceleration).

The general resistance of the body can be raised by amitetravit by means of activation of the functional state of the adreno-cortical system, increase of the hematopoietic base, intensification of the potential of hematopoietic cells for reproduction, activation of the function of the reticulo-endothelial system, immunobiological reactivity and stimulation of protein synthesis.

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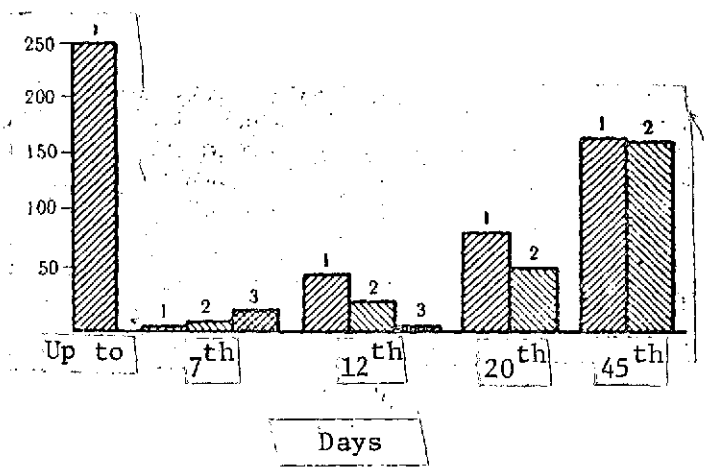

Figure 54. Changes in the total number of myelokariocytes in punctates of bone marrow of dogs subjected to automyelotherapy. Symbols same as Fig. 53.

We must, evidently, assume the chief pathway of the anti-radiation effect of ATP is its participation in processes of biosynthesis, disturbed because of a lack of macroergic compounds. Stimulation of the formation of endogenic macroergs can be assumed under the effect of ATP. The participation of ATP is also possible in intra-cellular recovery processes, in particular, in reparation of potential damages to the chromosomes [72, 61]. This is indicated by the reduced frequency of chromosome rearrangements in bone marrow cells as the

result of prophylactic administration of ATP to mice in cases of brief radiation [22]. This can be connected with the greater preservation and more rapid recovery of the number of cells in hematopoietic organs in animals protected by ATP.

There is basis for assuming that ATP to a certain degree can prevent damages to unique genetic structures, primarily DNA, which underly the mutagenic effect of radiation. This can be related to the ability of ATP to cause symptoms of hypoxia in hematopoietic tissues [22].

We must also not fail to consider the possible positive effect of ATP on the post-radiation recovery of unique cellular structures, as it is greatly dependent on the intensity of oxidation-reduction processes, ATP synthesis and the related microenergy balance.

The high effectiveness of the combined use of ATP and amitetravit in tests on dogs indicates, in our opinion, that this direction of studies searching for anti-radiation agents among drugs able to prevent primary damages to genetic structures and accelerate processes of reparation in the first stages of the pathological process will open prospects for further purposeful theoretical and practical works.

First it was shown that automyelotransplantation as a method of pathogenetic treatment of acute radiation sickness can be successfully used not only with brief, but also with prolonged radiation simulating powerful chromospheric solar flares. As this method is completely safe in relation to the development of immunological complications and, moreover, at the present time reliable means are being developed for the prolonged preservation of bone marrow, the feasibility of creating a bone marrow bank for cosmonauts and other persons possibly encountering random radiation must probably be considered. /220

In conclusion it must be noted that the joint works of scientists of a number of countries in the "Interkosmos" program have validated the use of a number of new methods of preventing and treating radiation injuries in accordance with the aims of space medicine.

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CONCLUSION

The calendar of the second half of the 20th century has already recorded /227
many remarkable dates of the space era. Only 17 years ago, on October 4, 1957, the world's first successful launch of an artificial Earth satellite was made in the USSR. Other satellites followed, realizing the ancient dream of mankind — to penetrate the depths of the Universe. Following the first space flight by Y. A. Gagarin, new important achievements were made in the exploration and mastery of space: man's penetration into deep outer space, exploration of the Moon with automatic space equipment, flights of automatic stations to planets in the solar system and landing on their surface, the first flights of man to the Moon, the creation of the first manned orbital stations.

The development of cosmonautics has had a significant effect not only on general scientific and technical progress, but also on all aspects of the life of modern man. Knowledge obtained as the result of space research has extraordinarily enriched the basic sciences, expanded the sphere of our world view and thought, encouraged broader philosophical concepts of natural phenomena and /228
positively affected the development of our ideas of the universe. Space exploration is of great help in the development of space biology and medicine. Important for the creation of long-term orbital stations and for future space flights is the regular and systematic accumulation of knowledge on the effect of unusual space flight factors on the human body and theoretical possibilities of living and working for a long time in space. Comprehensive solution of urgent medico-biological problems assisted in devising scientifically-based recommendations regarding the selection, preparation and training of crews, the creation of an optimum regime of work, rest, nutrition and daily life of cosmonauts, medical control of their health in flight, physiological validation of artificial atmosphere parameters in the manned cabin and radiation safety of flights.

The global nature of space research has naturally caused the development of international space and technical cooperation and helped it in every possible way. The successful solution of many medico-biological problems of space flights required not only the inclusion of trained scientific forces within each country, but also cooperation with scientists of other countries.

The present collective monograph generalizes the first summaries of five years of joint work in space biology and medicine by specialists of socialist countries, carried out in the "Interkosmos" program. The book gives results of joint studies on space physiology, radiation safety of space flights and pharmaco-chemical protection against ionizing radiation.

Oriented toward the exploration and mastery of a peaceful outer space, cosmonautics recognizes its purpose to serve peace. As complex studies conducted by scientists of socialist countries on space biology and medicine have developed and deepened, it has become evident that the results of these works also help introduce them into national public health practice.

Materials of the book indicate that space science has not only penetrated the sphere of experimental-theoretic research conducted in clinical and sports medicine, but also is being used successfully in the diagnosis, treatment and prevention of diseases.

We are confident that the present work will help the development of medicine, including space medicine, and will contribute to the solidarity and expansion of professional communication between scientists of socialist countries working in the field of the exploration and use of outer space for peaceful purposes. These high aims in combination with a friendly and creative atmosphere will provide every possiblity for successful joint solution of urgent medico-biological problems in the "interkosmos" program.